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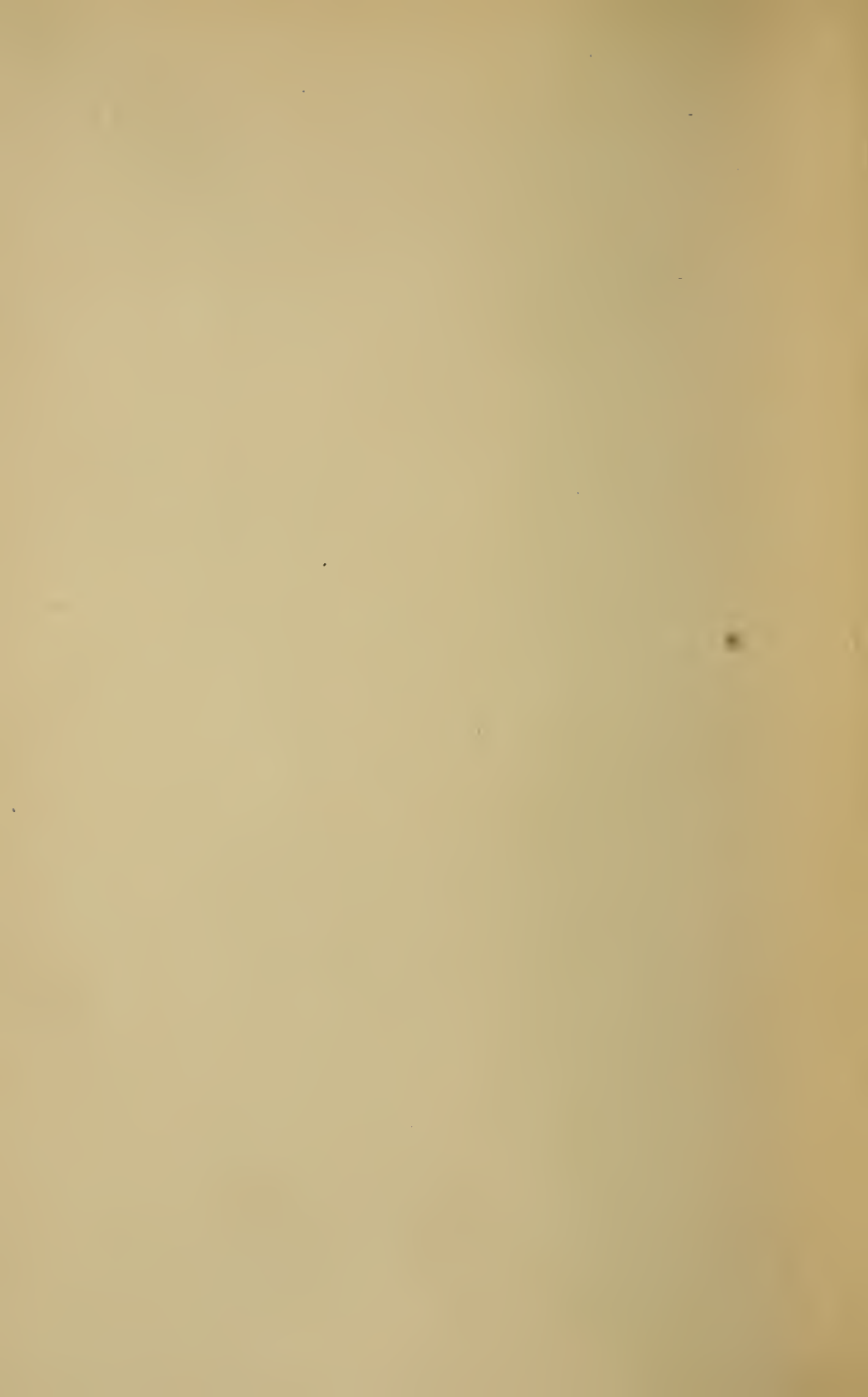
GIES FELLOWSHIP

*in Biological Chemistry*









HANDBOOK  
FOR THE  
BIO-CHEMICAL LABORATORY.

INCLUDING  
METHODS OF PREPARATION  
AND  
NUMEROUS TESTS  
*ARRANGED ALPHABETICALLY.*

BY  
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the City of New York.*

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## PREFACE.

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IN this little handbook an attempt has been made to give concise directions for preparing the most important substances that enter into the composition of the fluids and tissues of the animal body. The methods herein presented are compiled from the most recent and important works on physiological chemistry; and in certain instances two or three procedures are given for obtaining the same result.

The two hundred or more tests are arranged in alphabetical order; and the name of the scientist who suggested the test, or the name under which it is ordinarily known, is given in each case. My most earnest desire in compiling this handbook has been both to facilitate general work in bio-chemical laboratories and to afford the student an opportunity to have conveniently at hand all the necessary facts in modern scientific testing, so that loss of time in consulting works of reference might be reduced to a minimum.

JOHN A. MANDEL.

COLLEGE OF THE CITY OF NEW YORK,

January, 1896.



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# HANDBOOK FOR BIO-CHEMICAL LABORATORY.

## Glycogen, $C_6H_{10}O_5$ .

*Preparation.*—1. Kill a large well-fed rabbit by cutting its throat, open the abdomen immediately and remove the liver. After weighing, cut it up in rather large pieces and quickly throw them in boiling water (about 400 c.c. to 100 grms. liver), and let boil for half an hour. Then remove the pieces and grind them up finely in a mortar, return to the boiling water and add caustic potash solution (3–4 grms. KOH to 100 grms. liver). Now warm on the water-bath, and allow it to concentrate until you have 200 c.c. of liquid for every 100 grms. liver. If a scum forms on the surface, place the liquid in a beaker, covering it with a watch-glass, and heat it until all has dissolved, then put aside to cool. Neutralize with HCl and precipitate the albuminous bodies by the alternate addition of HCl and a solution of potassio-mercuric iodide \* (*Brücke* reagent) in small portions. The addition of potassio-mercuric iodide must be continued until no further precipitate occurs. If the liquid at last remains milky, nearly neutralize with caustic soda, and then treat with HCl again. Filter off the precipitate of albuminous bodies through thick filter-paper, and wash by removing the precipitate from the filter by means of a spatula and place it with water containing HCl and

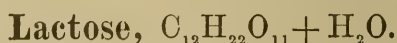
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\* This solution is prepared by saturating a boiling, not too concentrated (10%) solution of potassium iodide with pure mercuric iodide and filtering after cooling.

potassio-mercuric iodide and then return to filter. This treatment must be repeated at least four times. The several filtrates are united and, while stirring, treated with 2 vols. 96% alcohol, which precipitates the glycogen, and allow it to stand in a cool place overnight. Filter off the precipitate and wash first with 62% and then with 98% alcohol. This glycogen generally contains but a trace of albumins, but if required more pure, dissolve it while still moist in a little warm water, add some HCl and potassio-mercuric iodide after allowing to cool, and proceed as above. Lastly, wash the glycogen, which has been previously treated with absolute alcohol, a couple times with ether, and allow it to dry in the air or over sulphuric acid. (*R. Kulz.*)

2. *Brücke's* method consists in precipitating the albuminous bodies from the watery extracts by HCl and potassio-mercuric iodide without previously extracting with caustic potash, and then proceeding as above directed.

*Properties.*—Glycogen is a white amorphous powder, easily soluble in hot water, yielding an opalescent solution, which when allowed to evaporate on the water-bath forms a pellicle over the surface which disappears again on cooling. The solution is dextro-rotatory, ( $\alpha$ )  $D = +211^\circ$  (*Kulz*). On boiling with dilute mineral acids, or by the action of diastatic enzymes (ptyalin, diastase), it is readily converted into maltose, isomaltose, and dextrose. Its solution does not reduce Fehling's solution on boiling, but holds copper oxyhydrate in solution in alkaline liquids. With a solution of iodine glycogen solutions are colored wine-red which disappears on heating. Glycogen does not ferment with yeast.



(MILK-SUGAR.)

*Preparation.*—The sweet whey obtained after the precipitation of casein from milk (see page 13) is heated to boiling,

filtered, evaporated to dryness with magnesium carbonate, and the residue extracted with alcohol. Exhaust the part insoluble therein with hot water, filter, and evaporate the filtrate to a syrupy consistency, and allow to stand in a cool place until the lactose crystallizes out. If the syrup is at all colored, the solution must be decolorized by passing the solution through animal charcoal.

*Properties.*—Lactose crystallizes in rhombic prisms which contain a molecule of water of crystallization. It is soluble in 6 parts cold and 2.5 parts hot water. It has only a faint sweet taste. It is insoluble in ether or absolute alcohol. Aqueous solutions are dextro-rotatory,  $(\alpha) D = +52.5^\circ$ . Milk-sugar combines with bases; the alkali combinations are insoluble in alcohol. Solutions of lactose reduce Fehling's solution, but less powerfully than dextrose. On warming with phenyl-hydrazine acetate it gives on cooling a yellow precipitate of phenyl-lactosazon,  $C_{24}H_{32}N_4O_9$ . By boiling with water, or more readily on boiling with acids, or by means of inverting ferments, as in the alimentary canal, it takes up water and is converted into a glucose called galactose. It undergoes alcoholic fermentation by the action of certain schizomycetes, producing lactic acid at the same time.

### Maltose, $C_{12}H_{22}O_{11} + H_2O$ .

*Preparation.*—500 grms. potato-starch are thoroughly mixed with 2.5 litres cold water and converted to a paste by heating on the water-bath. Allow this paste to cool to  $60-65^\circ C$ ., and add a watery extract of 30–35 grms. of air-dried malt made at  $40^\circ C$ . Keep at  $60^\circ C$ . for an hour, then boil, filter, and evaporate to syrup in a flat porcelain dish. This is extracted several times with boiling 90% alcohol. If no crystals of pure maltose are at your disposal, boil a portion of the syrup with absolute alcohol, filter, evaporate to thin syrup, and allow it to stand in thin layers until it crystallizes, which



generally takes place in a few days. In the meantime distil most of the alcohol off from the main portion, evaporate the residue to a thick syrup, and on cooling stir into this a few crystals of the pure crystallized maltose. After three to five days the syrup will have crystallized to a stiff mass of crystals. These are rubbed to a thin paste with methyl alcohol, drained on paper, and washed once with methyl alcohol, pressed, washed again with methyl alcohol, and purified by further crystallization. For this purpose dissolve 500 grms. of the dried, pressed maltose in 15 c.c. water on the water-bath, add 130 c.c. 90% alcohol, boil, filter, and allow to cool. No syrup should separate out. Add a few crystals of pure maltose, and shake often, until the entire liquid after a few hours crystallizes into a thick mass of crystals. After draining the crystals they may be recrystallized from methyl alcohol, which is done by heating 50 grms. of the crystals with 12 c.c. water until all has dissolved, and adding 300 c.c. methyl alcohol; boil, filter, and allow to cool. Shaking facilitates crystallization considerably. (*Soxhlet.*)

*Properties.*—Maltose crystallizes generally in microscopic needles containing 5% (1 mol.) water of crystallization. The dried crystals are hygroscopic, specific rotatory power being ( $\alpha$ )  $D = +137^\circ$ . Maltose reduces alkaline solutions of copper, bismuth, and other metallic salts, but its reducing power as measured by Fehling's solution is  $\frac{1}{3}$  less than that of dextrose. With phenyl-hydrazine acetate it gives after heating for  $1\frac{1}{2}$  hours clusters of yellow crystals,  $C_{24}H_{32}N_4O_9$ , melting at  $206^\circ C$ . Maltose is easily and completely fermented by yeast. When heated with very dilute sulphuric acid, maltose yields dextrose. The diastatic enzymes act in the same way.

### Dextrose, $C_6H_{12}O_6$ .

*Preparation.*—Warm a mixture of 1.5 litres 90% alcohol and 60 c.c. strong HCl on the water-bath to  $45^\circ C$ , and



gradually add 500 grms. powdered cane-sugar, stirring all the while and taking care that the temperature does not rise above 50° C. After two hours the sugar will have dissolved and will be inverted into dextrose and lævulose ; allow it to cool, and place it in a cold place until crystallization commences, which occurs in from six to eight days. The crystallization may be facilitated by constant stirring. If pure anhydrous crystals of glucose are at hand, add a few grammes to the cold solution and stir well. In this case the crystallization will begin in a few hours, and is complete in 36 hours. The crystals thus obtained are well drained, then washed free from HCl by 90% alcohol, then with absolute alcohol, and now dried at a moderate heat. To completely purify these crystals boil them for five to ten minutes with pure methyl alcohol\* (sp. gr. 0.810 at 20° C.), filter, quickly cool, and the glucose crystals will separate out. (*Soxhlet*).

*Properties.*—Dextrose (glucose, grape sugar) is readily soluble in water, sparingly soluble in alcohol, and insoluble in ether. It crystallizes from an aqueous solution in white spheroidal masses, and from alcohol in transparent anhydrous prisms. Its solutions rotate the ray of polarized light to the right ; ( $\alpha$ )  $D = + 52.6^\circ$ . In alkaline solutions dextrose reduces salts of silver, bismuth, mercury, and copper. Under the influence of yeast it is converted into alcohol and carbon dioxide. It may also undergo lactic-acid fermentation under the influence of certain bacterial growths. With a mixture of 2 parts phenyl-hydrazine hydrochloride and 3 parts sodium acetate a watery solution of glucose gives, when heated on the water-bath, a precipitate of fine yellow needles (phenyl glucosazon,  $C_{18}H_{22}N_4O_4$ ), melting at 204°–205° C.

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\* The purest methyl alcohol is mixed with about 20% water, and about four-fifths distilled off on the water-bath. This distillate has, as a rule, the above specific gravity, and is immediately used.

### Inosit, $C_6H_{12}O_6$ .

*Preparation.*—1. Make a watery extract of 2 lbs. chopped meat, remove the albuminous bodies by coagulating at boiling heat. This is filtered and the filtrate precipitated by sugar of lead, and again filtered and washed. This filtrate is boiled with basic lead acetate and allowed to stand 24–48 hours. The precipitate thus obtained, which contains all the inosit, is decomposed in water by  $H_2S$ . The filtrate is strongly concentrated, treated with 2–4 vols. hot alcohol, and the liquid removed as soon as possible from the tough and flaky masses which ordinarily separate. If no crystals separate from the liquid within 24 hours, then treat with ether until the liquid has a milky appearance and allow it to stand. In the presence of a sufficient quantity of ether, crystals of inosit separate within 24 hours. The crystals thus obtained, as also those which are obtained from the alcoholic solution directly, are recrystallized by redissolving them in very little water and the addition of 3–4 vols. alcohol.

2. Inosit may also be prepared from green beans by evaporating the watery extract to a syrupy consistency and precipitating with alcohol. The precipitate is dissolved in water and the inosit allowed to crystallize out. (*Vohl*.)

*Properties.*—Inosit crystallizes in large, colorless, rhombic crystals of the monoclinic system, or, if not pure and if only a small quantity crystallizes, it forms fine crystals similar to cauliflower. The crystals melt at  $217^\circ C$ . It dissolves in 6 parts water at the ordinary temperature, and the solution has a sweetish taste. It is insoluble in strong alcohol and in ether. Inosit does not ferment with beer yeast, but is capable of lactic-acid fermentation. It dissolves copper oxyhydrate in alkaline solutions, but does not reduce on boiling. It gives negative results with MOORE'S or BOETGER-ALMEN'S bismuth test. Its solutions have no action on polarized light. Inosit gives no combination with phenyl-hydrazin acetate.

## Fatty Acids, $C_nH_{2n}O_2$ .

*Preparation.*—Dissolve 20 grms. caustic potash in 100 c.c. absolute alcohol, placing the vessel in cold water as considerable heat is generated. When all has dissolved that will, decant the clear solution from the sediment. Now heat on water-bath 50 grms. mutton tallow or leaf lard with 50 c.c. alcohol in a flask connected with a return condenser. Continue the application of heat until all the fat has melted; now add the potash solution, and gently boil for one half to one hour. When the liquid in the flask does not give a cloudiness when added to water, then all the fat has been converted into soap. Filter through a cotton plug and dilute this liquid with 500 to 600 c.c. water. Boil over the naked flame until all the odor of alcohol has disappeared, and add dilute sulphuric acid (1 to 4) until the solution has a marked acid reaction. Allow this to stand on the boiling water-bath until the separated fatty acids have collected on the surface as an oily layer. Now allow to cool, filter through a wet filter, wash the fatty acids with cold water, and crystallize the same from hot 80% alcohol. The oleic acid ( $C_{18}H_{34}O_2$ ) remains nearly entirely in the mother liquid, while the palmitic ( $C_{16}H_{32}O_2$ ) and stearic acid ( $C_{18}H_{36}O_2$ ) forms the crystals. Determine the melting-point of the mixture, then dissolve the same in cold alcohol, and fractionally precipitate this solution with an alcoholic solution of sugar of lead (3 to 4 fractions are sufficient). Each precipitate is shaken with ether and the fatty acid obtained on the evaporation of the ether. Determine the melting-point of each fraction, and a different melting-point will be found for each, showing that the fatty acids obtained from the fat consists of a mixture. The first lead precipitate contains the stearic acid.

Stearic acid melts at  $69.2^{\circ} C$ .

Palmitic acid melts at  $62^{\circ} C$ .

## Soap.

*Preparation.*—Dissolve 50 grms. fatty acids (page 7) in 100 c.c. alcohol by warming on the water-bath. Gradually add an alcoholic solution of caustic soda (10 grms.  $\text{NaHO}$  in 100 c.c. alcohol) to this solution until a very faint alkaline reaction is obtained. Heat on water-bath for 15 minutes, transfer to flat porcelain dish, and evaporate off the alcohol on the water-bath. When nearly all alcohol is off, add 30 c.c. water and continue the evaporation, stirring all the while. The product thus obtained when dry will be a neutral soap.

## Serum Albumin.

*Preparation.*—1. Defibrinated ox blood (or human transfusions) is filtered through washed linen (free from starch) and allowed to stand in the cold in a tall vessel until the red blood-corpuscles have settled to the bottom. The clear serum is carefully drawn off by means of a siphon and saturated at  $30^{\circ}\text{C}$ . with magnesium sulphate, filtered at the same temperature, and washed with a saturated solution of magnesium sulphate. Saturate the filtrate with sodium sulphate (or ammonium sulphate) at  $40^{\circ}\text{C}$ ., whereby the serum albumin is precipitated. This precipitate is collected on a filter, pressed between paper, dissolved in water, reprecipitated by sodium sulphate (or ammonium sulphate), and the process repeated several times. The solution in water is now freed from salts by means of dialysis, using large amounts of distilled water. The serum albumin may be obtained from this dialyzed solution by evaporating the solution to dryness at a gentle heat, or, better, by precipitating with an excess of strong alcohol, filtering, washing with alcohol, and finally with ether, and then drying by exposure to the air.

When precipitating the serum albumin by means of alcohol filter immediately, press between paper, and remove the alcohol from the precipitate by means of ether.



2. Serum albumin may also be precipitated from the filtrate, after the precipitation of the serum globulin, by means of acetic acid—about 1%. Filter after a few hours, press the precipitate between filter-paper, dissolve in water, neutralize by the addition of alkali, and remove salts by means of dialysis. The serum albumin is obtained from this salt-free solution as above directed.

*Properties.*—In the dry state serum albumin forms a transparent, gummy, brittle, hygroscopic mass or a white powder, readily soluble in water, forming a clear solution with a specific rotatory power, for a solution saturated with NaCl, of (a)  $D = -62.6^{\circ}$  to  $64.6^{\circ}$ . The coagulation temperature is  $+70^{\circ}$  to  $75^{\circ}$  C., but varies with the varying concentration and the amount of salts. Its solutions are precipitated by alcohol and ether.

### Ov-Albumin.

*Preparation.*—1. The white of several hen's eggs (free from yolk) is subdivided by cutting with a scissors or by beating violently, filtered through linen (free from starch), and then treated with an equal amount of water. A precipitate will form, and this must be removed by filtration. Saturate the filtrate at  $20^{\circ}$  C. with very finely powdered magnesium sulphate ( $\text{MgSO}_4 + 7\text{H}_2\text{O}$ ), which is done by adding small portions of the salt at a time and constantly stirring. After completely saturating, remove the precipitated globulins by filtration, and thoroughly dialyze the filtrate until a portion removed and treated with  $\text{BaCl}_2$  does not give any reaction for sulphates. Evaporate the solution (which greatly increases in volume during dialysis) at  $40^{\circ}$ – $50^{\circ}$  C. in a flat dish, and allow this concentrated solution to undergo dialysis again; and, lastly, evaporate to dryness at the above-mentioned temperature.

2. Ov-albumin may also be obtained by saturating the fil-

trate from the magnesium sulphate with sodium sulphate (or ammonium sulphate) at  $20^{\circ}$  C. The precipitate of ov-albumin is filtered off and pressed between filter-paper, dissolved in water, again precipitated with sodium sulphate (or ammonium sulphate), and after repeating this process several times the salts are removed by dialysis, and the salt-free solution evaporated to dryness at  $40^{\circ}$  C., or in a vacuum.

*Properties.*—The ov-albumin remains as a yellowish, transparent mass soluble in water, precipitated from its solutions by alcohol, and quickly converted into coagulable albumin. It is not precipitated from its watery solution by  $\text{MgSO}_4$ , but completely precipitated by  $\text{NH}_4\text{SO}_4$ . Its 1–3% solution containing some salt coagulates at about  $56^{\circ}$  C. It is not precipitated by ether, and has a specific rotatory power of ( $\alpha$ )  $D = -35.5^{\circ}$ .

### Serum Globulin or Paraglobulin.

1. Faintly acidify blood serum (prepared as directed on page 8) with a few drops acetic acid, and dilute with 10–20 vols. of water. The serum globulin will separate as a fine flocculent precipitate, which is filtered and further purified by dissolving it in a dilute common-salt solution or in water by the aid of the smallest possible amount of alkali, and then reprecipitating by diluting with water or by the addition of a little acetic acid. On repeating this twice the serum globulin is carefully dried in the air. (*Al. Schmidt.*)

2. Serum may also be precipitated from blood serum by means of magnesium or ammonium sulphate added to saturation. Filter and wash with one-half saturated solution of the salt used, and purify the precipitate by means of dialysis. As ammonium sulphate is removed by dialysis with difficulty, it is best to use magnesium sulphate. When great purity is required the precipitate may be redissolved by adding distilled water, reprecipitating by saturating with the salt again, and purifying this by thorough dialysis. (*Hammarsten.*)

*Properties.*—Serum globulin is insoluble in water, but soluble in dilute salt solutions ( $\text{NaCl}$ ,  $(\text{NH}_4)_2\text{SO}_4$ ,  $\text{MgSO}_4$ ). Its solution in dilute solutions of  $(\text{NH}_4)_2\text{SO}_4$ , or  $\text{MgSO}_4$ , are completely precipitated by saturating these solutions with the respective salt, but it is incompletely precipitated by  $\text{NaCl}$ . The coagulation temperature with 5–10%  $\text{NaCl}$  in solution is  $75^\circ \text{C}$ . Specific rotatory power for a solution containing salt is (a)  $D = -47.8$ .

### Fibrinogen.

*Preparation.*—Precipitate salt plasma with an equal volume of a saturated solution of  $\text{NaCl}$  (33%). The precipitate thus obtained is pressed between filter-paper, redissolved in an 8% salt solution, the filtrate precipitated by a saturated salt solution as above, and after precipitating in this way three times, the precipitate at last obtained is filtered, pressed between filter-paper, and finely divided in water. These operations should be performed rapidly, as prolonged contact with a half-saturated salt solution renders the precipitate of fibrinogen very insoluble. The fibrinogen dissolves by the aid of the small amount of  $\text{NaCl}$  contained in itself, and the solution may be made salt free by dialysis with very faintly alkaline water. (*Hammarsten.*)

*Properties.*—Fibrinogen has the general properties of the globulins, namely, insoluble in water, but soluble in dilute neutral salt solutions. It is precipitated unchanged from these solutions on sufficiently diluting with water. On heating it coagulates. When dissolved in a 5–10%  $\text{NaCl}$  solution it coagulates at  $52^\circ$  to  $55^\circ \text{C}$ , and the faintly alkaline or nearly neutral weak solution coagulates at  $56^\circ \text{C}$ . Its specific rotatory power for sodium light is  $-52.5^\circ$ .

### Myosin.

*Preparation.*—1. Finely chopped meat is extracted by 5% magnesium-sulphate solution. The filtered extract is then

treated with  $\text{MgSO}_4$  in substance until 100 c.c. of the liquid contains about 50 grms. of the salt. The so-called paramyosin or muscudin separates. The filtered liquid is now treated with magnesium sulphate until each 100 c.c. of the liquid holds 94 grms.  $\text{MgSO}_4$  in solution. The myosin which now separates is filtered, dissolved in water by the aid of the retained salt, precipitated by diluting with water, and, when necessary, purified by redissolving in dilute salt solution and precipitating with water. (*Halliburton.*)

2. Myosin may also be prepared by treating finely chopped meat, which has first been soaked in cold water until the muscles are white, with a 10–20% ammonium-chloride solution, allowing to stand for a few hours, stirring now and then. Filter this solution off and dilute with about 20 vols. water. The myosin separates in flakes which gradually settle to the bottom. This is washed 3 to 4 times by decantation, but not oftener, as then the myosin becomes insoluble. Dissolve now in ammonium-chloride solution and the myosin obtained therefrom, either by reprecipitating by diluting with water, or by removing the salt by dialysis. (*Danilewsky.*)

*Properties.*—Myosin has the general properties of the globulins. It is completely precipitated by saturating with  $\text{NaCl}$ , also by  $\text{MgSO}_4$ , in a solution containing 94% of the salt with its water of crystallization. Like fibrinogen it coagulates at  $56^\circ \text{C}$ . in a solution containing common salt, though the coagulation temperature may vary for myosins of different origin, and also for the same myosin in different salt solutions. It is soluble in dilute alkalies. Myosin decomposes hydrogen peroxide.

### Ovo-Vitellin.

*Preparation.*—Shake the yolks of two eggs with 200 c.c. acid-free ether in a stoppered cylinder, then add 5 c.c. alcohol. A sticky, stringy precipitate will be formed. Remove the ether as well as possible and add 100 c.c. of a 10% common salt



solution. On shaking the precipitate it dissolves in the common salt solution, yielding a cloudy liquid; place the solution in a separatory funnel and shake with an equal volume of ether. It will remain clear or nearly so. Draw off the watery solution and allow it to stand until the next day, when a cloudiness will have appeared; this is settled by re-shaking again with ether. Draw off the watery liquid again, measure it, and dilute with 10 vols. of water. The very fine precipitate produced is filtered off the following day, washed with water and then with alcohol. To further purify the substance thus obtained place the precipitate in a flask, boil with absolute alcohol on the water-bath, filter, wash with alcohol, then with ether, and lastly subdivide the mass in a flat dish and allow to dry over sulphuric acid or in a vacuum. (*Salkowski.*)

*Properties.*—Vitellin is insoluble in water, but soluble in dilute neutral salt solutions. It is soluble in hydrochloric acid of 1 p.m. and in very dilute solutions of alkalies or alkali carbonates. The coagulation temperature for the solution containing NaCl lies between 70° and 75° C. It yields nuclein when digested with pepsin and hydrochloric acid. Vitellin obtained as above should contain only 0.95% phosphorus.

## Casein.

*Preparation.*—200 c.c. fresh milk are diluted with 800 c.c. water and treated with acetic acid so that the dilute milk contains 0.75–1 p.m. acetic acid. (Hydrochloric acid may also be used.) The casein hereby precipitated is quickly washed with water by decantation and rubbed with water in a mortar so that it is as fine as possible. Dissolve it with the least possible quantity of a 0.1% caustic-soda or ammonia solution, continually stirring, and taking care that the liquid does not become alkaline but neutral. The milk-white liquid is filtered through several folds of filter-paper, when it will become water-clear

with only a slight bluish opalescence. After diluting with water it is again precipitated by acetic acid (or hydrochloric acid) as above directed, and the precipitate again ground finely, washed on a filter with water, and then dissolved in caustic soda as above. This is repeated once or twice. Then the washed precipitate is gently pressed, quickly rubbed to a paste with 97% alcohol, transferred to a filter, washed first with alcohol, then with ether, pressed, then dried in a mortar after having finely divided it. The last traces of ether are removed in a vacuum or over sulphuric acid. (*Hammarsten.*)

*Properties.*—Casein forms a white, dusty, insoluble powder which reddens moist blue litmus-paper. It is readily soluble in dilute alkalies and acids. It is completely soluble in 0.2% hydrochloric acid, and if this is digested at 38–40° C. with pepsin a gradual cloudiness is formed and a precipitate of nuclein is produced. Casein solutions do not coagulate on boiling, but are covered, as milk, with a skin. Casein is precipitated from neutral solutions or from milk by NaCl or  $\text{MgSO}_4$  in substance without changing its properties. Metallic salts, such as copper sulphate, completely precipitate casein from neutral solutions. Casein coagulates with rennet or chymosin in the presence of lime-salts.

### Albuminate (Alkali).

*Preparation.*—Beat up the white of an egg finely and filter through a piece of clean linen, and treat the filtrate with a solution of 1 grm. caustic potash in a little water, continually stirring. The solution will be immediately, or after some time, converted into a gelatinous mass. This is cut into pieces, washed a few times with water, then dissolved in warm water, allowed to cool, and precipitated by acetic acid, washing the precipitate with alcohol and ether. This precipitate of alkali albuminate appears as a flaky, amorphous, white substance, nearly insoluble in water as well as NaCl solutions,

but readily soluble in alkalies  $\text{Na}_2\text{CO}_3$ ,  $\text{Na}_2\text{HPO}_4$ , as well as dilute hydrochloric acid. It does not coagulate on applying heat to its solutions.

### Albuminate (Acid).

*Preparation.*—Digest the white of two eggs with hydrochloric acid (0.5%), and let it stand or apply gentle heat; then dilute with twice its volume of water. Collect the precipitate, dissolve it in hot water, and carefully neutralize the solution with  $\text{Na}_2\text{CO}_3$ ; finally wash well with water, and the product will be pure acid albuminate.

### Fibrin,

*Preparation.*—Whip freshly-drawn ox-blood with a bunch of twigs; the fibrin adheres to the twigs and entangles but a few blood-corpuscles. The mass is washed for a long time in a stream of running water until nearly white, and then with a 5% common salt solution, and again with water. When free from  $\text{NaCl}$  extract with alcohol and then with ether, and preserve in a solution of equal parts glycerine and water.

*Properties.*—Fibrin is soluble with difficulty in a 5–10% common salt or saltpetre solution or similar solutions of  $\text{MgSO}_4$  or other neutral salts. In the presence of enzymes or by putrefaction it may dissolve. It is insoluble in water, alcohol, or ether. Fibrin decomposes hydrogen peroxide. Solutions of fibrin are precipitated by lead acetate, copper sulphate, and mercuric chloride. Weak  $\text{HCl}$  (0.2%) causes fibrin to swell up into a transparent jelly, while stronger acids dissolve it in a time with the formation of acid albumin or syntonin and albumoses.

## Peptone.

*Preparation.*—1. Digest 2000 grms. washed fibrin, but not boiled, with 5 litres of a solution of purified pepsin (see Pepsin) obtained from 600 grms. of the removed mucous membrane of the fundus of the stomach of two pigs and containing 0.4% HCl. This mixture is allowed to digest for a fortnight at 37°–40°C., so as to insure as complete a conversion of the albumoses into peptones as possible. A little thymol (0.25%) may be added to prevent putrefaction. The formation of peptones takes place much more quickly with trypsin digestion; therefore it may be used instead of pepsin. After complete digestion the solution is filtered through linen and should not contain any albuminates or coagulable albumin. Exactly neutralize the filtrate with soda and heat to boiling. While boiling hot precipitate by saturating with ammonium sulphate. Allow to cool, and separate the precipitated albumoses and the ammonium sulphate, which has crystallized out, by filtration. The filtrate is again heated to boiling, and made strongly alkaline with ammonia and ammonium carbonate and saturated with ammonium sulphate while boiling. Filter when cold, and boil the filtrate until the odor of ammonia has entirely passed off; now saturate again with ammonium sulphate while hot, and acidify with acetic acid. When cold filter and strongly concentrate the filtrate, stirring constantly, and when cold decant the liquid from the salts which have crystallized out. A great part of the remaining ammonium sulphate may be removed by careful fractional precipitation with alcohol ( $\frac{1}{2}$  vol.), so that at last a solution rich in peptones, containing alcohol and some little ammonium sulphate, is obtained. The alcohol is driven off by boiling the solution, and the ammonium sulphate decomposed by boiling with barium carbonate. The filtrate is freed from the excess of barium by the careful addition of dilute sulphuric acid. This last filtrate, which must not contain any sulphuric acid, is



strongly concentrated, and the peptones precipitated by the addition of alcohol. The peptones thus obtained are quickly dried in a desiccator connected with an air-pump. (*Kühne*.)

2. The solution obtained after the digestion of fibrin with pepsin as described in method 1 is first neutralized with sodium hydrate, filtered through linen, and the filtrate acidified with acetic acid and concentrated considerably. Now precipitate the albumoses by saturating with ammonium sulphate, filter and press, and boil the solution with barium hydrate, and finally with barium carbonate and a large quantity of water, until ammonia can no longer be detected. Remove the barium sulphate by filtration through cloth bags, and the filtrate evaporated after washing the precipitate. The barium peptone thus obtained is decomposed by a slight excess of sulphuric acid, the barium sulphate filtered off, the solution concentrated, the free acid neutralized with ammonia, and after cooling add 6% sulphuric acid (previously diluted). Now precipitate with a large excess of phospho-tungstic acid, filter, wash first with 6% sulphuric acid, then with a large quantity of water, after which the compound is decomposed by means of barium hydrate and the excess of barium completely removed from the filtrate by the careful addition of sulphuric acid. The solution of peptones thus obtained has a distinct acid reaction, and the solid peptone may be obtained therefrom by repeated precipitation and boiling with alcohol. (*Kühne* and *Chittenden*.)

## Nuclein.

*Preparation*.—1. Pure casein is dissolved in water containing 2 p.m. HCl and filtered, and the filtrate digested with pepsin at 40° C. After a time a precipitate of nuclein appears; this is filtered off, washed with warm water, and purified by repeated solution in a 1% solution of sodium carbonate and reprecipitated after filtration by the addition of dilute hydro-

chloric acid. The nuclein thus obtained is washed with water and extracted with alcohol and then ether. Dry over sulphuric acid.

2. Suspend some beer-yeast in water and wash by decantation, and then add it to water containing 5 p.m. HCl. After some time add a slight excess of soda. Filter immediately through a rapid filter and allow to flow into dilute HCl, when a precipitate will fall to the bottom of the flask. Wash by decantation first with dilute hydrochloric acid, then with water, and then with boiling alcohol. Dry in a vacuum.

*Properties.*—The nucleins are colorless, amorphous, insoluble or very slightly soluble in water. They are insoluble in alcohol and ether. They are more or less readily soluble in alkalis; in dilute mineral acids they are insoluble or dissolve with difficulty. On boiling with caustic alkali they decompose and alkali phosphates are formed. On fusing with soda and saltpetre they give alkali phosphates also.

### Nucleinic Acids.

*Preparation.*—Treat 1000 c.c. well-washed yeast with 3250 c.c. of a 3% caustic-soda solution, and allow to digest at the temperature of the room for 5 minutes. Neutralize with hydrochloric acid, and add an excess of acetic acid. Filter off the precipitated albuminous bodies and measure the filtrate. Now add HCl to filtrate so that it contains 3–5 p.m., and then add an equal volume alcohol which has previously been acidified to the same extent (3–5 p.m.). The impure nucleinic acid thus precipitated is filtered off, dissolved in ammoniacal water, and purified by treating as above, namely, acetic acid, hydrochloric acid, and alcohol. The purified product is dried in desiccator. (*Altmann.*)

*Properties.*—Nucleinic acids are white, amorphous, and acid in reaction, readily soluble in ammoniacal or alkaline water, and are not precipitated therefrom by an excess of

acetic acid, but readily by a slight excess of hydrochloric acid, especially in the presence of alcohol. They are insoluble in alcohol and ether.

### Lecithin.

*Preparation.*—The yolk of several eggs free from white are violently shaken with ether until the ether does not dissolve any more. The several ethereal extracts are freed from ether by distillation, and the residue dissolved in petroleum ether and filtered. The filtrate is transferred to a separatory funnel and shaken several times with 75% alcohol; the alcoholic extracts are united, and allowed to stand until entirely clear. Separate any petroleum ether present, and filter. The last portions of petroleum ether are distilled off, and the residue is allowed to stand in a cold place for several days, when a precipitate will be produced. The liquid is separated from the precipitate by decantation and then filtration. Decolorize the solution by boiling with animal charcoal, filter, and evaporate to syrupy consistency at 50–60° C. Dissolve the syrup in ether, filter, and evaporate the filtrate, when the lecithin will be obtained in a nearly pure form. To further purify, dissolve in as little absolute alcohol as possible, and on cooling this solution in a freezing mixture to –5° to –15° C. the lecithin will be deposited. This is filtered while cold, and dried in a vacuum over sulphuric acid. (*E. Gilson.*)

*Properties.*—Lecithin is a soft, waxy, mouldable, not markedly crystalline mass, which when placed on a microscope slide with water forms oily drops, appearing under the microscope like worms, called “myeline forms.” It is easily decomposed by acids and alkalis, especially by the latter. On fusion with soda and saltpetre on platinum foil it yields a mass in which phosphoric acid can be easily detected. It combines with acids and bases. The combination with HCl gives a double salt with  $\text{PtCl}_4$ , which is insoluble in alcohol,

but soluble in ether, and which contains 10.2% platinum. It is soluble in chloroform, carbon disulphide, benzol, and fatty oils.

### Oxyhæmoglobin.

*Preparation.*—Defibrinated blood (best from the dog or the horse) is mixed with at least 10 times its volume of a salt solution (1 vol. saturated solution with 9 vols. water), and allowed to stand in a cool place for a couple of days until all the blood-corpuscles have settled to the bottom. Remove the supernatant liquid by means of a siphon, and transfer the blood-corpuscles to a flask. Add 2 vols. water, and then shake with an equal volume of ether. After a time remove the ether by decantation, and allow the ether retained by the blood solution to evaporate in an open dish in the air. Filter quickly through a folded filter, cool the solution to  $0^{\circ}$  C., and add while stirring  $\frac{1}{4}$  of its volume of alcohol which has been cooled to  $0^{\circ}$  C. and allow to stand for several days at  $-5^{\circ}$  to  $-10^{\circ}$  C. The crystals which separate are filtered off, pressed, and recrystallized by first quickly dissolving them in not too much water at  $20^{\circ}$  to  $30^{\circ}$  C., cooling to  $0^{\circ}$  C., precipitating with cold alcohol as above, and allowed to stand at  $-5^{\circ}$  to  $-10^{\circ}$  C. This recrystallization is repeated several times. Lastly, the crystals are washed with cooled water containing alcohol ( $\frac{1}{4}$  vol. alcohol), and dried in vacuum at  $0^{\circ}$  C. or a lower temperature. (*Hoppe Seyler.*)

*Properties.*—Oxyhæmoglobin from various kinds of blood has different crystalline forms. It is insoluble in ether, chloroform, benzol, and carbon disulphide. Crystals of oxyhæmoglobin are insoluble in absolute alcohol. Its solution in water is not precipitated by many metallic salts, but is precipitated by sugar of lead. On heating the watery solution it decomposes at  $60^{\circ}$ – $70^{\circ}$  C., and splits into hæmatin and albumin. It is also decomposed by acids, alkalies, and many metallic salts.



Dilute solutions show a spectrum with two absorption-bands between the Fraunhofer lines *D* and *E*. The one band,  $\alpha$ , which is narrower but darker and sharper, lies on the line *D*; the other, broader, less defined and less dark band,  $\beta$ , lies at *E*. Oxyhæmoglobin solutions are reduced by reducing solutions such as ammonium sulphide, ammoniacal ferro-tartrate solution (*Stokes* reduction liquid) yielding a characteristic spectrum of hæmoglobin.

### Hæmoglobin.

*Preparation.*—Fill a cylinder nearly full with a dilute watery solution of oxyhæmoglobin, and add a few drops of a putrefied extract of meat. Close up hermetically, and allow to stand some time at the ordinary temperature. The oxyhæmoglobin will be reduced to hæmoglobin, and if the solution is of the proper concentration a crystallization of hæmoglobin may occur in the cylinder at lower temperatures. The solution of hæmoglobin is more purple than a solution of oxyhæmoglobin, and shows a spectrum with only one absorption-band occupying the space between the Fraunhofer lines *D* and *E*.

### Carbon-monoxide Hæmoglobin.

*Preparation.*—Pass a current of carbon monoxide (prepared by heating a mixture of sulphuric and oxalic acids and passing the gases through a wash-bottle containing KOH), through a watery solution of oxyhæmoglobin. The color of the solution becomes lighter, due to the formation of carbon-monoxide hæmoglobin. The spectrum of the solution is not changed, but it differs from the oxyhæmoglobin in that it cannot be reduced by reducing solutions into hæmoglobin. The crystals are isomorphous to the oxyhæmoglobin crystals, but are less soluble.

## Methæmoglobin.

*Preparation.*—On treating a concentrated solution of oxy-hæmoglobin with a sufficient quantity of a concentrated solution of potassium ferricyanide to give the mixture a porter-brown color, methæmoglobin will be formed. After cooling to  $0^{\circ}$  C. add  $\frac{1}{4}$  vol. alcohol cooled to  $0^{\circ}$  C., and allow the mixture to stand a few days in the cold. The crystals obtained may be easily purified by recrystallization from water by the addition of alcohol. (Pig's blood is better adapted for the preparation of methæmoglobin than dog's blood.)

*Properties.*—Methæmoglobin crystallizes in brownish-red needles, prisms, or six-sided plates. It dissolves readily in water, and this solution becomes red on the addition of alkali. The spectrum of methæmoglobin shows three absorption-bands, one in the red about half way between *C* and *D*, the other two between *D* and *E*, which resemble the position of the oxyhæmoglobin lines, but on careful measurement are found to be different.

## Fibrin Ferment.

*Preparation.*—Blood-serum is mixed with 10 to 15 times its volume of absolute alcohol; by this means the proteids are precipitated, as also the ferment. The precipitate is allowed to stand a few months with the alcohol, which renders the proteids insoluble. Filter the precipitate off and dry in desiccator over sulphuric acid. The ferment is separated from the other bodies by extracting with water, which dissolves the ferment. (*A. Schmidt.*)

*Properties.*—A solution of the ferment will coagulate pericardial and similar fluids or solutions of fibrinogen. It is most active at about  $40^{\circ}$  C. It is diminished in action at  $0^{\circ}$  C., and entirely destroyed on heating its solutions to  $73^{\circ}$ – $75^{\circ}$  C.

**Glycocholic Acid,  $C_{26}H_{43}NO_6$ .**

*Preparation.*—1. The precipitate obtained by treating an alcoholic solution of dried decolorized bile with ether is dissolved in water, and dilute sulphuric acid added until a permanent and dense turbidity is produced. Allow this liquid to stand a few hours, when glycocholic acid will separate out as fine silky needles. Collect on filter, wash with water, press between folds of filter-paper, and then dissolve in alcohol, using as little alcohol for solution as possible. Add many times its volume of ether, and glycocholic acid will separate out as long silky needles.

2. Decolorized extract of ox-bile is dissolved in water and the solution treated with a solution of lead acetate. A precipitate of lead glycocholate is produced. Collect on a filter, wash, drain, and mix with alcohol. To this add a solution of sodium carbonate in excess and evaporate to dryness. This converts the lead glycocholate into sodium glycocholate. Remove the sodium glycocholate from the lead glycocholate by extracting the residue with absolute alcohol. Distil off the alcohol nearly to dryness, and evaporate to dryness in a porcelain dish. Dissolve this residue in water, decolorize this solution by animal charcoal, and the glycocholic acid is precipitated from the solution by the addition of dilute sulphuric acid. The acid thus obtained is purified by crystallization from boiling water on cooling, or by the addition of ether to its solution in absolute alcohol.

3. Fresh ox-bile is treated with a few drops HCl, which causes the precipitation of the mucoid substances. Filter, and to each 100 c.c. filtrate add 5 c.c. strong HCl. Place in a stoppered cylinder and add 30 c.c. ether for every 100 c.c. bile, shake well, and allow to stand in a cool place. After a time the mixture is found to be converted into a mass of crystals of glycocholic acid. Filter and wash with ice-water until the washings are colorless; they are then dissolved in

the smallest possible quantity of boiling water, which deposits, on cooling, crystals in a colorless and pure state. (*Hüfner*.)

*Properties*.—Glycocholic acid crystallizes in fine, colorless needles or prisms. It is soluble with difficulty in water (about 300 parts cold and 120 parts boiling water). Readily soluble in strong alcohol, but with greater difficulty in ether. The solutions have a bitter taste, but at the same time a sweetish taste. The salts of the alkalies and alkaline earths are soluble in alcohol and water, while the glycocholates of the heavy metals are either insoluble or only sparingly soluble in water. It is a monobasic acid. The solution of the alkali-salts in water is precipitated by lead acetate, copper, ferric salts, and silver nitrate. On boiling with acids or alkalies it splits into cholalic acid and glycocoll. Glycocholic acid or its salts are dextro-rotatory. Specific rotation of the acid dissolved in alcohol is (*a*)  $D = +29^\circ$ .

### Taurocholic Acid, $C_{26}H_{45}NSO_7$ .

*Preparation*.—Agitate dog's bile with animal charcoal and alcohol, allow to settle, decant through filter. The clear filtrate is evaporated to dryness and dissolved in a little hot absolute alcohol and precipitated by the addition of ether. This crystalline precipitate of sodium taurocholate is filtered, dissolved in water, and precipitated by lead acetate and ammonia, filtered, washed well, and then boiled with absolute alcohol, filtered while hot, and the filtrate decomposed with  $H_2S$  until no further precipitation of  $PbS$  occurs. This is filtered, the filtrate concentrated by evaporation at a gentle heat and then precipitated by an excess of water-free ether. The precipitate consists of taurocholic acid which after a time becomes converted into needle-shaped crystals possessing a silky lustre. If the precipitate refuses to crystallize, after a time add a drop or two of alcohol. (*Parke*.)

*Properties*.—Taurocholic acid crystallizes in needles which



rapidly deliquesce in the air. It is readily soluble in water and alcohol, yielding a solution with a bitter-sweetish taste. Its salts are, as a rule, readily soluble in water, and the solutions of the alkali salts are not precipitated by copper sulphate, silver nitrate, or sugar of lead, but are precipitated, on the contrary, by basic lead acetate. The aqueous solution of the alkali salts foam like soap. On boiling with acids and alkalies it splits into cholalic acid and taurin. Both aqueous and alcoholic solutions of taurocholic acid are dextro-rotatory. Specific rotation of its alcoholic solution is (a)  $D = +25^{\circ}$ .

### Cholalic Acid, $C_{24}H_{40}O_6$ .

*Preparation.*—1. 500 c.c. bile are treated with 75 grms. barium hydrate and the mixture boiled for 24 hours on the sand-bath. The flask should be connected with a return condenser. Allow the liquid to cool, and filter. Add concentrated hydrochloric acid to the filtrate, which decomposes the barium cholalate, depositing impure cholalic acid. These crystals are separated from the mother-liquor and pressed, and dissolved in a solution of caustic soda; the solution is then mixed with 30 grms. animal charcoal and allowed to stand for a few days. It is next filtered and the filtrate decomposed with hydrochloric acid, filtered again and washed thoroughly and the substance from the filter dissolved by the smallest possible quantity of hot alcohol. The alcoholic solution is treated with water until a slight turbidity appears. On the liquid being cooled cholalic acid separates in the form of hard, transparent tetrahedra or in clumps of radiating needles. For further purification recrystallize from methyl alcohol. (*Mylius.*)

2. Mix ox-bile with one fifth of its weight of a 30% caustic-soda solution and boil for 24 hours on water-bath, having the flask connected with a return condenser. Now saturate the liquid with  $CO_2$  and evaporate to dryness. Extract the residue with strong alcohol, which dissolves the sodium salts

of cholalic acid as well as those of choleic and stearic acids. Now dilute with water until it does not contain more than 20% alcohol, and add a dilute solution of barium chloride as long as a precipitate occurs. Filter, and add to the filtrate, which should yield no further precipitate with  $\text{BaCl}_2$ , hydrochloric acid. Allow the precipitate of cholalic acid to stand with the liquid and it will be found to become crystalline; purify by recrystallization from ethyl alcohol and then from methyl alcohol. (*Mylius*.)

*Properties*.—Cholalic acid crystallizes with 1 molecule of water in rhombic plates or prisms, or in larger rhombic tetrahedra or octahedra with 1 mol. alcohol of crystallization. They are quite insoluble in water (4000 parts cold and 750 parts boiling), rather soluble in alcohol, but soluble with difficulty in ether. It is somewhat soluble in glycerin and almond-oil. Its solutions have a bitter-sweetish taste. The water-free acid melts at  $195^\circ \text{C}$ . In the free state, as well as in combination the acid is dextro-rotatory. The specific rotatory power of the sodium salt is (*a*)  $D = +31.4^\circ$ . The watery solution of the alkali-salts, when not too dilute, is precipitated immediately or after some time by sugar of lead or by barium chloride. The barium salt,  $(\text{C}_{24}\text{H}_{39}\text{O}_6)_2\text{Ba}$ , crystallizes in fine, silky needles, which are soluble in 30 parts cold and 23 parts boiling water, but more soluble in alcohol.

### Glycocoll or Glycocine, $\text{C}_2\text{H}_5\text{NO}_2$ .

*Preparation*.—1 part hippuric acid is boiled from 10 to 12 hours, in a flask connected with a return condenser, with 4 parts dilute sulphuric acid (1 to 6). After allowing to cool pour the contents of the flask into a porcelain dish and allow to stand for 24 hours. Filter off the benzoic acid and wash with cold water; concentrate the filtrate by evaporation, and shake with ether to remove any traces of benzoic acid. Dilute this acid solution and just neutralize with barium

hydrate, which precipitates the sulphuric acid; allow the precipitate to settle, wash by decantation with boiling water, and concentrate the solution by evaporation. If an excess of  $\text{Ba}(\text{OH})_2$  has been added, remove this by passing  $\text{CO}_2$  through the solution, boiling and then filtering, and allowing to stand until crystals commence to form. The crystals are removed from the mother-liquor and this concentrated further, and the process repeated as long as crystals continue to separate out. The glycocoll is purified by recrystallization from water.

*Properties.*—Glycocoll occurs as fine, hard, colorless, rhombic crystals or four-sided prisms. The crystals have a sweetish taste, are soluble in 4.3 parts cold water, very slightly soluble in spirits of wine, and insoluble in cold absolute alcohol and ether. It combines with acids, bases, and neutral salts. Glycocoll dissolves copper hydroxide in alkaline solution, but does not reduce at the boiling temperature, but deposits on cooling dark blue needles if the liquid is sufficiently concentrated. Its solutions possess an acid reaction. When heated to  $232^\circ$ – $236^\circ$  C., glycocoll becomes brown, evolves gas-bubbles, and melts. A solution of glycocoll is colored red by ferric chloride. Its combination with  $\text{HCl}$  is soluble in water and alcohol.

### **Taurin, $\text{C}_2\text{H}_7\text{NSO}_3$ .**

*Preparation.*—Mix ox-bile with strong hydrochloric acid so as to precipitate the mucoid substances, filter, and boil the filtrate for several hours. After allowing to cool and to deposit the dyslysin and cholidic acid concentrate the liquid on the water-bath, filter so as to remove any common salt or other substances which may have separated. The filtrate is treated with strong alcohol, which precipitates the taurin with some  $\text{NaCl}$ . This precipitate is washed with alcohol, dried, and dissolved in the smallest quantity of boiling water possible. On cooling the taurin deposits in fine four-sided prisms which may be further purified by recrystallization from water.

*Properties.*—Taurin crystallizes in colorless, often large, shining, 4–6-sided prisms, which dissolve in 15–16 parts water at the ordinary temperature, but rather more readily in warm water. It is insoluble in alcohol and ether. Taurin is tasteless and its solutions are neutral to test-paper. On boiling with strong caustic alkalies it yields acetic acid and sulphurous acid but no alkali sulphides; but this decomposition does not occur on boiling with HCl or HNO<sub>3</sub>. Taurin combines with metallic oxides such as mercuric oxide. Metallic salts do not precipitate solutions of taurin.

**Bilirubin**, C<sub>16</sub>H<sub>18</sub>N<sub>2</sub>O<sub>3</sub> or C<sub>32</sub>H<sub>36</sub>N<sub>4</sub>O<sub>6</sub>.

*Preparation.*—1. Finely-powdered gallstones (of oxen) are first extracted with ether and then with boiling water to remove cholesterin and bile-acids. The residue is treated with dilute hydrochloric acid, which sets the pigment free. The insoluble powder is thoroughly washed with water and alcohol and then dried and boiled with chloroform until entirely extracted. Evaporate the chloroform solution on the water-bath and treat the powdered residue with absolute alcohol and ether, which removes the *bilifuscin*. Dissolve the remaining residue, insoluble in alcohol, in chloroform; precipitate the bilirubin from this solution by the addition of alcohol, and repeat this several times. The bilirubin is finally dissolved in chloroform and allowed to evaporate spontaneously, when crystals of bilirubin separate.

2. Dilute bile (preferably from the dog) with water, and precipitate with milk of lime. Pass carbon dioxide through the mixture, filter and wash. Suspend the precipitate in water, decompose it with hydrochloric acid and shake with chloroform, care being taken to avoid an excess of air. The chloroform solution is evaporated to a very small volume, precipitated by alcohol, and purified by repeated solution in chloroform and reprecipitating with alcohol.



*Properties.*—Bilirubin occurs partly amorphous and partly crystalline, as rhombic plates whose obtuse angles are often rounded. It is insoluble in water, almost insoluble in ether, and very sparingly soluble in alcohol; easily soluble in chloroform, especially in the warmth, and less soluble in benzol, carbon disulphide, amyl alcohol, fatty oils, and glycerin. Its solutions show no absorption-bands and have, even on diluting greatly (1 to 50,000), a decided yellow color. The combinations with alkalies are insoluble in chloroform. Bilirubin forms compounds with bases such as sodium, calcium, barium, lead, and silver in ammoniacal solution. On allowing an alkaline solution of bilirubin to stand in contact with the air it gradually absorbs oxygen, and green biliverdin is formed.

**Biliverdin,**  $C_{16}H_{18}N_2O_4$  or  $C_{32}H_{36}N_4O_8$ .

*Preparation.*—1. Biliverdin is readily prepared by exposing a thin layer of an alkaline solution of bilirubin to the air or by passing a current of oxygen through the alkaline solution until it has acquired a bright green color. Add hydrochloric acid, which precipitates the biliverdin, filter, and wash the precipitate with water until no reaction for HCl is obtained in the filtrate. Dry the precipitate, dissolve in alcohol, and separate the pigment by the addition of water to the alcoholic solution. Any bilirubin present may be removed by extracting with chloroform.

2. Add  $PbO_2$  to an alkaline solution of bilirubin and stir. The liquid assumes a dark green color in a few minutes; now acidify with acetic acid, which precipitates a compound of biliverdin with lead. This combination is decomposed by means of alcohol containing sulphuric acid in solution. The alcoholic solution containing the biliverdin is separated from the lead sulphate by filtration and the filtrate poured into water, which precipitates the biliverdin. Collect the precipitate on a filter

and purify by repeated solution in alcohol and reprecipitating with water.

*Properties.*—Biliverdin is amorphous, but it has occasionally been obtained as green rhombic plates with tunicated ends. It is insoluble in water, ether, pure chloroform, benzol, but readily soluble in ethyl alcohol, methyl alcohol, glacial acetic acid, and in chloroform containing alcohol. It dissolves in alkalies, yielding a brownish-green solution which is precipitated by acids, as well as by calcium, barium, and lead salts. Solutions of biliverdin exhibit no definite absorption-bands with the spectroscope. An absorption occurs from the red toward the violet end of the spectrum.

### Cholesterin, $C_{26}H_{44}O + H_2O$ .

*Preparation.*—Powdered gallstones are first boiled with water and then repeatedly boiled with alcohol. The solution is filtered quickly through a heated filter, and the cholesterin separates out in a fairly pure condition as the filtrates cool. The crystals are washed with cold alcohol and then boiled with an alcoholic solution of caustic soda, which saponifies the fats present. After the evaporation of the alcohol, extract the cholesterin from the residue by means of ether, which dissolves the cholesterin, but not the soaps; filter and evaporate the ether, and purify the cholesterin by recrystallization from a mixture of alcohol and ether.

*Properties.*—Cholesterin crystallizes from anhydrous ether or chloroform as needles containing no water of crystallization, but from alcohol as rhombic transparent plates whose sides and angles often appear broken and whose acute angle is  $76^{\circ} 30'$  or  $87^{\circ} 30'$ , with 1 mol. water of crystallization. It melts at  $145^{\circ} C$ . when free from water of crystallization. Cholesterin is insoluble in water, dilute acids, and alkalies. It is readily soluble in boiling alcohol; also in ether, glycerin, chloroform, and benzol, as well as the volatile and fatty oils. Its solutions

are lævo-rotatory, the specific rotatory power being (a)  $D = -31.6^\circ$ .

### Ptyalin.

*Preparation.*—1. Mixed human saliva is strongly acidulated with phosphoric acid and the mixture carefully neutralized by the careful addition of lime-water, which produces a copious precipitate of calcium phosphate, and which carries down with it a large proportion of the proteids present, together with all the ptyalin. On extracting the precipitate, after filtration, with a volume of water equal to that of the saliva originally employed, the enzyme passes into solution and is obtained therefrom by the addition of absolute alcohol, which precipitates it in the form of white flocculi, which when dried in vacuo appears as a white powder. (*Cohnheim.*)

2. Saliva is diluted with an equal volume of water and saturated with ammonium sulphate. The precipitate thus formed is treated on the filter for 5 minutes with strong alcohol, removed from the filter, and further treated with absolute alcohol for 1-2 days. It is now dried at  $30^\circ \text{C.}$  and yields, on extraction with a volume of water equal to that of the original saliva, a solution which is actively zymolytic and free from proteids. (*Krawkow.*)

### Pepsin.

*Preparation.*—1. The mucous coat of the pig's stomach is scraped with a watch-glass, and after carefully washing and drying, by pressing between filter-paper, it is finely minced. The mass is now digested with a 5% solution of  $\text{H}_3\text{PO}_4$  at  $35^\circ \text{C.}$  until nearly all is dissolved. Nearly neutralize the filtered solution with lime-water, which causes a precipitate of calcium phosphate, and which carries down with it the greater part of the pepsin previously in solution. The peptones and para-peptones remain in solution. The gelatinous precipitate is carefully washed with water, pressed between filter-paper,

suspended in water, and treated with HCl until it just dissolves. The solution is then poured into a saturated solution of cholesterin, made by dissolving it in a mixture of 4 parts alcohol and 1 part ether. A precipitate of cholesterin will be formed on the addition of the above acid solution, and this is repeatedly shaken with the liquid, and the cholesterin will mechanically carry down with it a part at least of the pepsin in solution. Collect this on a filter, wash with water, then with acetic acid, and lastly with water again until the wash-water gives no further reaction for HCl with  $\text{AgNO}_3$ .

The moist cholesterin precipitate is shaken in a separatory funnel with ether. Two layers will form—the upper an ethereal solution of cholesterin and the lower an aqueous solution of pepsin. Separate the two and shake the aqueous solution repeatedly with ether until all traces of cholesterin are removed. The turbid aqueous solution is now filtered, and when acidulated possesses proteolytic activity. When allowed to evaporate spontaneously it leaves a grayish, amorphous, non-hygroscopic nitrogenous body. (*Brücke.*)

2. The finely divided mucous membrane of the fundus of the stomach is extracted with glycerin, or, better, with glycerin containing 1 p.m. HCl. Add 10 to 20 parts glycerin for every part by weight of the mucous coat and allow to stand for 8 to 14 days. Filter and precipitate the pepsin from the glycerin extract by the addition of a large excess of absolute alcohol. This may be purified by dialysis through parchment paper. (*v. Wittich.*)

### Artificial Gastric Juice.

*Preparation.*—1. The mucous membrane from the cardiac region of five or six pigs' stomachs is finely divided and then digested for two weeks, at  $40^\circ \text{C.}$ , with two to three litres of 0.5% hydrochloric acid. At the end of this time all but traces of albumoses have been converted into peptones, which are in



solution together with pepsin, although a small portion of foreign matters, nucleins, antialbumid, etc., remain undissolved. The liquid is filtered and saturated with powdered  $(\text{NH}_4)_2\text{SO}_4$ , when a precipitation of the albumoses with the pepsin occurs. The precipitate is collected on a filter, washed with a saturated solution of ammonium sulphate, and then dissolved in 0.2% HCl. Add 0.25% thymol to this acid solution and dialyze in running water until the whole of the  $(\text{NH}_4)_2\text{SO}_4$  is removed. On opening the dialyzing tubes a precipitate is found, which is soluble in 0.2% HCl and furnishes a very active gastric juice. (*Kühne and Chittenden.*)

2. The mucous coat of a freshly removed pig's stomach is carefully washed with water and finely divided, or, if a calf's stomach is employed, only the cardiac region is scraped with a watch-glass or the back of a knife. The pieces of the mucous coat or the slimy masses obtained by scraping are rubbed up with pure quartz sand or glass powder, treated with water, and allowed to stand for 24 hours in a cool place. On filtering, an opalescent liquid is obtained, which, when acidulated so as to contain 0.1–0.2 per cent HCl, possesses powerful digestive activity. (*Kühne.*)

3. The stomach of a pig is opened, emptied, and then the surface cleaned with a wet sponge (running water will dissolve out a considerable part of the pepsin). The mucous membrane is removed from all but the pyloric end of the organ. It is then freed from a portion of the water adhering to it by pressing between dry cloths and then mincing. The finely divided mucous membrane is then placed in two or three litres of dilute HCl containing from 6 to 10 c.c. strong HCl per litre, and the mixture is digested in an incubator at a temperature of  $35^{\circ}$ – $45^{\circ}$  C. for a period varying from a few hours to a day. If sufficient fluid be present and the mixture now and then shaken, all ought to be dissolved in a few hours, leaving but a small quantity of brownish flakes and some mucus undissolved. The liquid is filtered through paper, and then may

be kept for several months without decomposition, retaining active proteolytic properties. Such juice does not, however, contain merely acid and pepsin, but considerable quantities of albumoses and peptones. (*Gamgee's Phys. Chem.*, vol. ii., page 82.)

## Milk-curdling Ferment of the Stomach.

(RENNIN OR CHYMOSIN.)

*Preparation.*—Cut up five calves' stomachs finely, after washing, and digest the mass for about 24 hours with a 0.5% common-salt solution, keeping the temperature at about 30° C., or less. After digestion filter, measure the filtrate, and acidify with hydrochloric acid, so that it contains 0.1% of the acid. A thick precipitate of mucous matter is formed, and to facilitate its easy separation the liquid is kept at 20°–30° C. and allowed to stand until all of the mucous matter has separated, or centrifugal force may be used. Filter, measure the filtrate, and acidify with HCl again so that the filtrate contains 0.5% acid, and now add powdered common salt until no more salt dissolves. This supersaturated, acidulated salt solution is brought to a temperature of 25°–30° C., and kept at this temperature for 2–3 days, under constant agitation, and then allowed to remain quiet for a day or so, the temperature being raised to 30° to 35° C. A flocculent substance is obtained, which rises to the surface of the liquid or is suspended therein. This is carefully filtered off, and then dried at a temperature of about 28° C. The product thus obtained is the pure zymotic product.

*Properties.*—Rennin or chymosin is an amorphous, white, gelatinous substance, greatly resembling aluminium hydrate. It is without taste or smell, soluble in water, forming a limpid or clear solution. Chymosin has the property of curdling large amounts of milk at 37° C., or pure casein solutions containing calcium salts.



## Trypsin.

*Preparation.*—1. The fresh pancreas of an ox is freed from adhering fat and connective tissue and then minced and digested first with cold alcohol, and afterwards extracted with boiling ether in an extraction apparatus. The insoluble residue is then exposed to the air, so as to allow the ether to evaporate, when there is left a white, friable mass. This may be kept indefinitely, and made use of to prepare solutions of trypsin. (Called *Kühne's* pancreas powder.)

One part by weight of the above pancreas powder is digested at 40° C. for 4 hours with 5 parts of 0.1% salicylic acid solution. The residue after being squeezed is further digested for 12 hours with 5 parts of 0.25%  $\text{Na}_2\text{CO}_3$  solution, and the residue is again squeezed out. The acid and alkaline extracts are mixed together, the whole made up to contain 0.25–0.5%  $\text{Na}_2\text{CO}_3$ , and digested for at least a week in the presence of 0.5% thymol. By this means all the first-formed albumoses are fully converted into peptones; this is essential. At the end of the week the fluid is allowed to stand in the cold for 24 hours, filtered, faintly acidulated with acetic acid, and saturated with neutral ammonium sulphate. By this means all the trypsin is separated out and may be collected on a filter, where it is washed with  $(\text{NH}_4)_2\text{SO}_4$  (saturated solution) till free from peptones. It is now finally dissolved off the filter in a little 0.25%  $\text{Na}_2\text{CO}_3$  solution, to which thymol is added, and thus an extremely active and pure digestive solution is obtained. The ammonium sulphate present may be gotten rid of by dialysis. (*Kühne*.)

2. The pancreas is carefully removed from a dog killed 18 to 20 hours after a full meal and weighed. It is then pounded up in a mortar with pure quartz sand or glass powder and allowed to stand at the temperature of the laboratory for 24 hours and then mixed with 1 c.c. of 1% acetic acid for every gramme of pancreas, then for each part of the gland mass add

10 parts glycerin and allow to stand for three days. After this time filter and precipitate the filtrate with alcohol and wash with alcohol. On now dissolving the precipitate in water we obtain a solution which has powerful digestive action. (*Heidenhain.*)

3. A watery infusion of the gland may be made only after it has been exposed to the air for 24 hours, adding 5 to 10 parts water for each part by weight of the gland. *Salkowski* suggests the digestion of the finely divided gland at 40° C. with water containing 5 to 10 c.c. chloroform per litre. After a few days we obtain by this means an active solution of trypsin which keeps.

*Properties.*—Trypsin is very soluble in water, but insoluble in alcohol and glycerin. Its watery solution is not decomposed by long digestion at 40° C., and when evaporated it yields a translucent, non-crystalline, yellowish solid residue. Trypsin may be digested for a long time at 40° C. with caustic soda without decomposing.

### Diastatic Ferment of the Pancreas (Amylopsin).

*Preparation.*—1. Finely divided pancreas, best after exposure for 24 hours to the air, is dehydrated first by being placed in strong alcohol and afterwards in absolute alcohol, the action of which should be continued for some time. The dry solid, separated from the alcohol, is then macerated in glycerin. The glycerin solution is precipitated by the addition of alcohol, filtered, washed with alcohol, dried over sulphuric acid, and then dissolved in water. (*v. Wittich.*)

2. Precipitate the aqueous infusion of a pancreas which has been treated with  $Mg_2CO_3$  with collodion, which carries the proteids and the proteolytic ferment down with it in a gelatinous form. The filtrate is concentrated by evaporation in *vacuo*, and treated with strong alcohol, which throws down a flocculent precipitate. Filter, and digest in a mixture

of equal parts alcohol and water, which dissolves the diastatic ferment, a little tyrosin, and some salts, leaving some albumin undissolved. The liquid is dialyzed, concentrated in *vacuo*, and precipitated by absolute alcohol. The precipitate thus obtained possesses feeble proteolytic properties, due to remaining traces of the proteolytic ferment, but has intense diastatic properties. (*Danielewski.*)

## The Fat-decomposing Ferment of the Pancreas

(Steapsin).

*Preparation.*—A perfectly fresh pancreas is crushed in a mortar with glass powder and mixed with a solution composed of 90 c.c. glycerin and 1 c.c. of a 1% solution of  $\text{Na}_2\text{CO}_3$ , using 30 c.c. of the glycerin solution for every 3 grms. of the pancreas. This is allowed to digest for not longer than four to five days and then removed from the mass of the pancreas. This extract has the property of decomposing neutral fats. (*Grützner.*)

**Leucin**,  $\text{C}_6\text{H}_{13}\text{NO}_2$ .

$\alpha$ . AMIDO-ISOBUTYL ACETIC ACID,

$(\text{CH}_3)_2\text{CH}.\text{CH}_2.\text{CH}(\text{NH}_2).\text{COOH}$ .

**Tyrosin**,  $\text{C}_9\text{H}_{11}\text{NO}_3$ .

*p*. OXYPHENYL-AMIDO PROPIONIC ACID,

$\text{C}_6\text{H}_4 \left\{ \begin{array}{l} p \text{ OH} \\ \text{CH}_2.\text{CH}(\text{NH}_2)\text{COOH} \end{array} \right.$

*Preparation.*—1. These two bodies may be prepared in large quantities by boiling albuminous bodies or albuminoids with dilute mineral acids. Two parts hoof-shavings ( $\frac{1}{2}$  to 1 kilo in weight) are boiled for 24 hours with 5 parts concentrated sulphuric acid and 13 parts water, adding water from time to time to replace that which has evaporated. After

boiling it is diluted with water and gradually treated with milk of lime with constant stirring until the liquid is neutral or a little alkaline. Filter through a folded filter, the residue on filter boiled with water several times, and after filtration added to the main filtrates. The several filtrates are concentrated by evaporation and oxalic acid added so as to precipitate all the lime; filter, boil the calcium oxalate precipitate with water several times, filter and unite all filtrates, evaporate until crystallization begins, and allow to cool. What first crystallizes out consists chiefly of tyrosin with only a little leucin. Filter this off and concentrate the liquid more, when a second crystallization will form on cooling, which consists of leucin with some tyrosin. This operation of evaporating and crystallizing is continued until no more crystals are obtained. The several batches of crystals are united and boiled with a large quantity of water and enough ammonia to dissolve them. To this hot solution add lead acetate, constantly stirring, until the precipitate formed is no longer brown, but white; filter, heat the yellow filtrate to boiling, neutralize or make faintly acid with dilute sulphuric acid, filter while boiling hot, and allow to cool. Pure tyrosin is deposited while leucin remains in solution.

The mother liquor from the tyrosin is treated with  $\text{H}_2\text{S}$ , filtered and concentrated and boiled a couple of minutes with freshly precipitated copper oxyhydrate. A deep blue solution is the result, which, if filtered and concentrated, deposits sky-blue crystalline warts, and an insoluble cuprous compound of leucin. This precipitate, as well as the crystalline warts, is decomposed in hot water with  $\text{H}_2\text{S}$ , filtered, and the filtrate decolorized when necessary with animal charcoal, strongly concentrated, and allowed to crystallize. The leucin which deposits may be purified by recrystallization from boiling alcohol or by precipitating it as leucin lead oxide, decomposing this precipitate when suspended in water with  $\text{H}_2\text{S}$ , and evaporating the filtrate to point of crystallization. The tyrosin may



be purified by recrystallization from boiling water or from ammoniacal water.

2. A large quantity of well-boiled fibrin is digested at 40° C. with a solution of *Kühne's* dried pancreas containing 1 per cent salicylic acid and some thymol. After a day or two the liquid is filtered, faintly acidified with acetic acid, and boiled. After filtration concentrate the filtrate nearly to syrupy consistency and set aside to cool. Considerable quantities of leucin and tyrosin will have crystallized out after 24 hours. Remove the crystals and concentrate the mother-liquor further if necessary, and precipitate the peptones with an excess of hot alcohol, and filter while hot. On cooling crystals of leucin will form if much is present. Pour off the mother liquor, and wash with a saturated solution of ammonium sulphate. The mixed leucin and tyrosin resulting from these operations must be separated and purified by the method of *Hlasiwetz* and *Habermann*, as described in method No. 1.

The leucin may be simplest separated from the tyrosin by boiling the yellow crystalline masses or crusts with alcohol, which dissolves the leucin and leaves the tyrosin in great part. After filtering and concentrating the filtrate leucin crystallizes out and is purified by repeated crystallization from alcohol. Tyrosin is obtained in a crystalline form by dissolving the residue insoluble in alcohol with water containing ammonia, and allowing this solution, after filtration, to evaporate at ordinary temperatures.

*Properties.*—Leucin crystallizes when pure in shining white, very thin, doubly refractive plates, nodular masses or balls, possessing a greasy feel and which float on water. Pure leucin dissolves in 27 parts cold water, and in 1040 parts cold and in 800 parts boiling alcohol, though the solubility seems to vary with the source of the leucin. On slowly heating it melts at 170° C., and sublimes in white, woolly flakes, which are similar to sublimed zinc oxide. Its specific rotatory power is (*a*)  $D = + 17.5^\circ$ . Solutions of leucin in water are not, as a

rule, precipitated by metallic salts. The boiling hot solution may, however, be precipitated by a boiling hot solution of copper acetate. It forms crystalline compounds with sulphuric, hydrochloric, and nitric acids.

Pure tyrosin crystallizes in colorless, silky, fine needles, which are often grouped in tufts or rosettes. From very impure solutions it separates in part or wholly in nodules and balls very like leucin. It is soluble with difficulty in water, being dissolved by 2454 parts water at  $20^{\circ}\text{C}.$ , and 154 parts boiling water. It is insoluble in alcohol and ether. Tyrosin is readily soluble in solutions of ammonia, caustic alkalies, and alkaline carbonates. It is also soluble in dilute mineral acids, with which it forms unstable compounds.

### Aspartic Acid, $\text{C}_4\text{H}_7\text{NO}_4$ .

#### AMIDO-SUCCINIC ACID, $\text{C}_2\text{H}_3(\text{NH}_2)(\text{CO.OH})_2$ .

*Preparation.*—The mother-liquor from which leucin and tyrosin have crystallized out (see page 38) is further concentrated and treated with a little alcohol, when, after some time, new crystalline crusts will separate. These are dissolved in water, and the solution boiled with freshly precipitated cupric hydrate. On filtering the blue solution will deposit the copper compound of aspartic acid, which occurs as light blue needles. These are dissolved in  $\text{HCl}$  and decomposed by  $\text{H}_2\text{S}$ , when white crystalline plates of aspartic acid will separate out. This is purified by crystallization from boiling water.

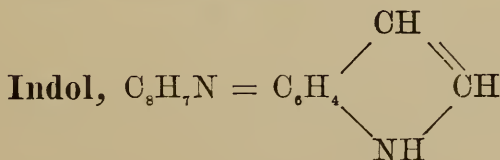
*Properties.*—Aspartic acid crystallizes in rhombic prisms which are sparingly soluble in cold water or alcohol, but readily soluble in boiling water. Its solution, strongly acid with nitric acid, is dextro-rotatory,  $(\alpha) D = +25.16^{\circ}$ , but in alkaline solution it is lævo-rotatory. With copper it forms a compound soluble in boiling water, but nearly insoluble in cold water.

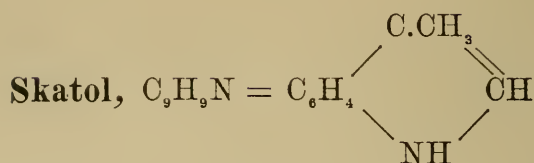


**Glutamic Acid,  $C_5H_9NO_4$ .**AMIDO-PYROTARTARIC ACID,  $C_5H_6(NH_2)(COOH)_2$ .

*Preparation.*—Boil 200 grms. casein (free from fat) with 600 c.c. concentrated  $HCl$  and 500 grms. stannous chloride for 3 days in a flask connected with a return condenser. After diluting with 10 vols. water remove the tin by means of  $H_2S$ , and evaporate to syrupy consistency on water-bath. On standing in the cold the syrup will solidify into a mass of crystals; these are stirred up and transferred to a filter connected with a filter-pump, and as much mother-liquor sucked off as possible; then place the moist mass on an unglazed tile and placed in desiccator until dry. Now recrystallize one or two times from as little water as possible with the addition of conc.  $HCl$ , and remove the mother-liquor as well as possible. The crystals are now dissolved in a great deal of water and boiled with an excess of lead oxyhydrate until the solution is free from chlorine. Filter, precipitate the lead by  $H_2S$ , filter again, and evaporate the solution to point of crystallization. If these crystals retain still small quantities of chlorine, treat the hot solution with some silver oxide or carbonate; filter, treat the filtrate with  $H_2S$ , filter, and evaporate to crystallization.

*Properties.*—Glutamic acid crystallizes in the form of small plates or rhombic tetrahedra or octahedra, which are sparingly soluble in cold water, but readily in hot water. It is insoluble in alcohol or ether, and melts at  $135^\circ$ – $140^\circ C$ . Its dilute  $HCl$  solution has a specific rotatory power of  $(\alpha) D = +31.1^\circ$  to  $31.6^\circ$ . Dilute solutions reduce Fehling's solution. When boiled with  $Cu(OH)_2$ , glutamic acid separates in the form of blue-colored prisms, which are soluble in 3400 parts cold and 400 parts boiling water.





or

METHYL INDOL.

*Preparation.*—Two kilogrammes of well-pressed blood fibrin are placed in a large flask (12 litres capacity) treated with 8 litres water (containing 2 grms.  $KH_2PO_4$  and 1 gm.  $MgSO_4$ ), and well mixed with 200 c.c. of a cold saturated solution of soda, then a few cubic centimetres of a putrefying infusion of meat with some fragments of the decomposed meat. The flask is closed with a stopper provided with a glass tube attached with a rubber tube to a wash-bottle half full of water. The rubber tube has a clamp which is left open during the first days of the experiment. The mass is digested at  $40^\circ$  to  $42^\circ$  C. for 10 days, the flask being shaken from time to time and the clamp closed as soon as the evolution of gas has ceased and only opened now and then so as to liberate the accumulated gases.

After this time the entire liquid contents of the flask is distilled off until the residue in the flask measures 1 to 1.5 litres. The strongly ammoniacal distillate is acidified with HCl and then precipitated by a solution of copper sulphate and filtered. The clear filtrate is thoroughly shaken with ether, which is best done by shaking fractions of  $\frac{1}{2}$  litre at a time in a separatory funnel drawing off the heavy liquid and adding new portions of the filtrate to the ether in the funnel, adding more ether from time to time. When one half of the filtrate has been extracted with ether distill the ether from the ethereal solution and use the ether for further extraction. The entire ethereal extracts are distilled until about 500 c.c. are left. This residue is thoroughly shaken 2 times with a solution of caustic soda to separate phenols and acids, and the ether now distilled off at the lowest possible temperature. The oily

residue, previously treated with some caustic-soda solution, is distilled in a current of steam\* until no more indol passes over.

The distillate is now shaken with ether, the ethereal solution distilled at the lowest possible temperature, and the residue allowed to evaporate in a deep vessel until, on being allowed to cool, it solidifies as a crystalline mass. The latter is then dried in a desiccator over sulphuric acid. (*E. and H. Salkowski.*) The crystals thus obtained, consisting of indol and skatol, are dissolved in a very small quantity of absolute alcohol and then treated with 8-10 vols. water. The skatol is precipitated, but not the indol. The skatol is filtered off and recrystallized from ether. The indol is obtained from the watery solution by extracting it with ether and allowing the ether to evaporate spontaneously.

The mass of crystals, consisting of indol and skatol, may be separated from each other by means of fractional distillation in a current of steam when the skatol passes over first.

Skatol may also be obtained from this mixture of crystals by dissolving about 0.5 gm. in a few drops of benzol and adding 1.5 gm. crystallized picric acid and sufficient benzol to dissolve all while being heated in a covered beaker-glass. On cooling the mass solidifies to a red crystalline mass. Now add 2 vols. petroleum ether and stir, filter after 24 hours, wash with petroleum ether, and allow the crystals to dry in the air. Place the crystals in a distilling flask and distill with dilute caustic soda, which decomposes the indol, while the skatol passes over and is condensed. This skatol is purified by recrystallization from ether.

*Properties.*—Indol crystallizes from hot aqueous solutions

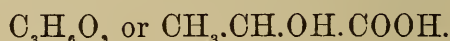
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\* The current of steam is best produced by filling a large flask two-thirds with water which has been acidified with a few cubic centimetres of dilute  $\text{H}_2\text{SO}_4$  and a few pieces of zinc, which by the action of the heat develops a gentle current of hydrogen, which prevents bumping and gives rise to a quiet, even generation of steam, which may be kept up for hours.

as small scales, which melt at  $52^{\circ}\text{C}$ . It is tolerably soluble in hot and less soluble in cold water; readily soluble in alcohol, ether, chloroform, benzol, and petroleum ether. It forms a combination with picric acid, consisting of red needles, which are decomposed on heating with caustic soda, but pass over without decomposition when distilled with ammonia. Indol has a peculiar excrementitious odor.

Skatol crystallizes in small plates, which melt at  $95^{\circ}\text{C}$ . It is less soluble in water than indol, but in the presence of steam it distils readily. It is readily soluble in alcohol, ether, chloroform, and benzol. With picric acid it forms a crystalline compound, which does not decompose on being heated with caustic soda, but passes unchanged into the distillate. It has an intense fetid odor.

### Ordinary or Fermentation Lactic Acid,



*Preparation.*—330 grms. cane-sugar and 0.5 gm. tartaric acid are dissolved in 1750 c.c. boiling water and allowed to stand for two days. Then add 4 grms. putrefying cheese (German Handkäse), 440 grms. sour milk, and 135 grms. zinc white, and allow this mixture to stand for eight to ten days at  $40^{\circ}\text{C}$ . with constant shaking. After this time heat to boiling, filter, and allow to cool, when zinc lactate will crystallize out. This is purified by recrystallization and then decomposed by  $\text{H}_2\text{S}$  in hot aqueous solution, the solution filtered, and the filtrate evaporated on the water-bath. The syrupy residue is dissolved in ether (to separate any mannite), and then the ether removed by distillation or by evaporation.

*Properties.*—Fermentation lactic acid has a similar appearance to sarcolactic acid, namely, a colorless, faintly yellowish, acid-reacting syrup, which mixes in all proportions with water, alcohol, or ether. It is optically inactive. It forms salts



readily, which are soluble in water and most of them in alcohol. The zinc salt dissolves in 58 to 63 parts of water at 14–15° C. and contains 18.18% water of crystallization, but is insoluble in alcohol. The calcium salt dissolves in 9.5 parts water and contains 29.22% (= 5 mol.) water of crystallization,

### Sarcolactic Acid,

PARALACTIC ACID,  $C_3H_6O_3$  or  $CH_3.CH(OH).COOH$ ,

*Preparation*.—Dissolve Liebig's extract of meat in 4 parts of water and then add 8 parts 90% alcohol, stirring all the while. The mixture is allowed to stand and settle, and the clear supernatant liquid is separated by decantation. To remove any lactic acid from the insoluble residue, mix it with twice its weight of warm water and precipitate by adding 4 or 5 times its volume of alcohol. The alcoholic solutions thus obtained are evaporated on the water-bath to a thin syrup, and this latter again precipitated by the addition of 3 or 4 times its volume of alcohol. The alcoholic solution is now evaporated to dryness, the residue mixed with water, some dilute  $H_2SO_4$  added, and then shaken several times with ether. On evaporating the ethereal solution a residue is obtained which consists of a mixture of sarcolactic and fermentation lactic acids.

To separate the two above acids they are dissolved in water and boiled with zinc carbonate or zinc white, the solution filtered, and the filtrate evaporated until crystals commence to form. The liquid is now treated with 4 or 5 times its volume of 90% alcohol; after some time the liquid becomes turbid and deposits needles, consisting of zinc sarcolactate, the zinc salt of the other acid remaining in solution. The crystals are collected on a filter, washed with absolute alcohol, dissolved in water, decomposed by  $H_2S$ , filtered, concentrated, shaken with ether, and the pure acid obtained on the evaporation of the ethereal solution,



*Properties.*—Sarcosine occurs as a syrup, which dissolves in water, alcohol, and ether readily. It is dextro-rotatory, but its salts, on the other hand, are lævo-rotatory. Its zinc salt crystallizes with 2 mol. (12.9%) water of crystallization as small four-sided prisms, which dissolve in 17.5 parts water, its calcium salt containing 24.83–26.21% (4 or 4.5 mol.) water of crystallization, and dissolving in 12.4 parts water.

### Protagon.

*Preparation.*—An ox-brain as fresh as possible, with the blood and membranes carefully removed, is ground fine and then extracted for several hours with 85% alcohol at 45° C., filtered while hot, and the residue extracted with warm alcohol until the filtrate fails to yield a precipitate at 0° C. The several alcoholic extracts are cooled to 0° C., and the precipitates united and completely extracted with cold ether, which removes cholesterin and lecithin-like bodies. The residue is strongly pressed between filter-paper and allowed to dry over sulphuric acid or phosphoric anhydride. The resulting mass is powdered, moistened with water digested for many hours with alcohol at 45° C., filtered, slowly cooled to 0° C. The crystals may be purified by recrystallization. (*Gamgee and Blankenhorn.*)

*Properties.*—Protagon appears when dry as a white, loose crystalline powder. It is soluble with difficulty in cold, but more easily soluble in warm, alcohol and ether. At temperatures above 55° C. its solutions decompose. It swells up in little water, decomposes partly, and gives myaline forms. It is soluble in glacial acetic acid, which deposits crystalline forms on cooling. When boiled with a solution of  $\text{Ba}(\text{OH})_2$ , protagon decomposes into glycerophosphoric acid, fatty acids, and neurine. It melts at 200° C., forming a brown syrup.

**Urea,  $\text{CH}_4\text{N}_2\text{O}$  or  $\text{H}_2\text{N.CO.NH}_2$ .**

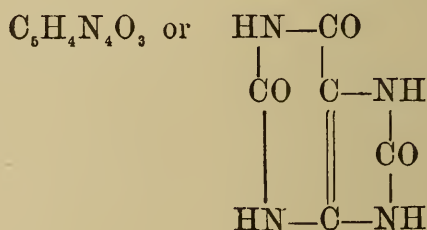
*Preparation.*—Evaporate  $\frac{1}{2}$  to 1 litre of urine to syrupy consistency on the water-bath, allow to cool in ice-water, treat while stirring with 3 vols. nitric acid of sp. gr. 1.3 which has previously been boiled and then cooled to  $0^\circ \text{C}$ . Allow this mixture to stand for a few hours at  $0^\circ \text{C}$ . The crystals of urea nitrate which form are transferred to a filter of glass wool and washed several times with small amounts of ice-cold pure concentrated nitric acid, then dissolved in as small a quantity of hot water as possible, and reprecipitated by conc.  $\text{HNO}_3$ . These crystals are first drained, then dissolved in hot water, and the solution decolorized by a little chlorine water, and then treated with a small quantity of pure barium carbonate until no more dissolves and effervescence ceases and the solution is neutral. Now evaporate the solution to complete dryness on the water-bath, pulverize the residue, and extract it with cold absolute alcohol, which dissolves the urea, but not the barium nitrate. The filtered alcoholic solution is decolorized when necessary by animal charcoal and evaporated at a medium heat to point of crystallization.

*Synthetical.*—Heat 250 grms. coarsely powdered potassium ferrocyanide in a capacious porcelain dish over a naked flame with constant stirring until all is changed into a white powder, leaving no lumps having a yellow nucleus. Care must be taken not to apply too strong a heat, as otherwise the powder will turn brown. The mass thus obtained is finely powdered and thoroughly mixed with one half ( $\frac{1}{2}$ ) its weight of dried finely powdered manganese dioxide, and the mixture heated on an iron dish under the draught, constantly stirring until deflagration begins and the mass becomes doughy. The heat is continued until a portion of the mass placed in water and acidified with  $\text{HCl}$  fails to give a blue precipitate with ferric chloride. Then allow to cool, lixivate with cold water, add to the solution  $\frac{3}{4}$  of the weight of the dried potassium

ferrocyanide of ammonium sulphate, filter, and evaporate at about  $60^{\circ}$ – $70^{\circ}$  C., at which temperature the ammonium cyanate is converted into urea. The crystals of potassium sulphate are removed from time to time and then the solution evaporated to dryness. The urea is obtained from this residue by extraction by absolute alcohol as above described.

*Properties.*—Urea crystallizes in needles or in long, colorless four-sided, often hollow, anhydrous rhombic prisms, similar to saltpetre. It has a neutral reaction, has a bitter taste, and produces a cooling sensation on the tongue. It melts at  $130^{\circ}$ – $132^{\circ}$  C., but partly decomposes at about  $100^{\circ}$  C. At ordinary temperatures it dissolves in equal weights of water and in five parts alcohol. Urea is readily soluble in amyl alcohol. It is insoluble in anhydrous ether, chloroform, and in petroleum ether. Urea combines with salts, such as NaCl,  $\text{NH}_4\text{Cl}$ , and the chlorides of the heavy metals, such as mercury, gold, zinc, copper, etc., producing crystalline combinations. It also combines with acids, both inorganic and organic, forming crystalline salts.

### Uric Acid,



*Preparation.*—1. Powdered Peruvian guano is just heated to boiling with 15–20 parts water in a porcelain dish, and then dissolved by the careful addition of a small amount of concentrated solution of caustic soda or potash, and now boiled until the odor of ammonia has disappeared. Filter and saturate the filtrate with carbon dioxide, when a gelatinous, afterwards granular, precipitate of acid potassium urate is

formed. The liquid is decanted after standing 24 hours, and the precipitate washed with small quantities of ice-cold water, and while still moist added to boiling dilute hydrochloric acid and kept boiling for some time, continually stirring. After cooling the separated uric acid is filtered, thoroughly washed with cold water, and dried. If not quite pure, it can be redissolved in alkali, precipitated by  $\text{CO}_2$ , and treated again as above, or it may be added to water and dissolved in the cold by the gradual addition of sodium amalgam, and after the removal of the impurities by filtration the uric acid may be reprecipitated by  $\text{HCl}$ .

2. Filtered normal urine is treated with 20–30 c.c. of 25%  $\text{HCl}$  for each litre of urine. After 48 hours collect the crystals and purify them by redissolving in dilute alkali, decolorizing with animal charcoal, and reprecipitating with hydrochloric acid. Filter, wash with ice-cold water, and dry.

3. Uric acid may be prepared synthetically by melting 0.1–0.15 grms. glycocoll with 1–2 grms. urea in a test-tube over a small flame. The mass is heated until solid, care being taken not to apply too strong (above  $220^\circ \text{C.}$ ) a heat, also not too low a heat. The cooled mass, which ought to be yellow or yellowish-brown, is dissolved in boiling water with the addition of ammonia and filtered; the filtrate is precipitated when cold with a mixture of magnesium mixture and ammoniacal silver nitrate solution. The precipitate is washed with ammoniacal water, subdivided in hot water, and treated with a solution of sodium sulphide. The filtrate from the silver sulphide is acidified with  $\text{HCl}$  and concentrated, when on cooling impure uric acid will crystallize out. Test these crystals with the murexid test, and if they do not give the reaction reprecipitate with ammoniacal silver solution and proceed as above, when comparatively pure uric acid will be obtained. It is better to make several fusions of glycocoll with urea, and adding these together and obtaining the uric acid therefrom in larger quantities. (*J. Horbaczewski.*)



*Properties.*—Pure uric acid is a white, odorless, and tasteless powder consisting of very small rhombical prisms or plates. It is insoluble in alcohol and ether, rather easily soluble in boiling glycerin, very difficultly soluble in cold water (14000–15000 parts), and difficultly soluble in boiling water (1800–1900 parts). It is soluble in sulphuric acid in the cold without decomposition, and readily soluble in many salts of the alkalis, as in the caustic alkalis; ammonia, however, scarcely dissolves it. Uric acid reduces alkaline solutions of copper or silver, but not alkaline solutions of bismuth.

### Hippuric Acid,



*Preparation.*—1. Treat fresh horse or cow urine with milk of lime until it is of a strong alkaline reaction; warm, filter, evaporate the filtrate to a syrupy consistency, and acidify strongly with HCl when cold. The hippuric acid thus precipitated is drained, washed with cold water, pressed between filter-paper, dissolved in as small a quantity of boiling water as possible, filtered, and the boiling-hot filtrate treated with chlorine gas until the color of the solution is pale yellow. Cool quickly, filter, wash the hippuric acid a few times with cold water, and crystallize from boiling water after treating the solution with animal charcoal. (*Th. Curtius.*)

2. One litre of the fresh horse or cow urine is made faintly alkaline with sodium carbonate and filtered, the filtrate evaporated nearly to dryness, and the residue repeatedly extracted with cold, strong alcohol. The alcohol is nearly entirely distilled off from the extracts the remaining watery solution acidified with HCl, and then repeatedly extracted with fresh portions of acetic ether (at least five times). The acetic ether extracts are united, and washed by shaking with water in a separatory funnel, and then evaporated at a moderate temperature. The residue is repeatedly treated with petro-



leum ether, which removes the benzoic acid, oxyacids, fat, and phenol, while the hippuric acid is left undissolved. The residue is dissolved in a little warm water, treated with animal charcoal, and evaporated at 50°–60° C. to point of crystallization. (*Bunge and Schmiedeberg.*)

*Synthetical.*—Hippuric acid may be prepared by adding a few drops caustic-soda solution to a watery solution of glycoll, shaking with benzoyl chloride, adding gradually an excess thereof and then making strongly alkaline with caustic soda. Now precipitate with hydrochloric acid on cooling. The precipitate of hippuric and benzoic acids are carefully drained, pressed between filter-paper and washed with cold water, dried, and extracted with petroleum ether, which only dissolves the benzoic acid. The remaining hippuric acid is recrystallized from hot water. (*J. Baum.*)

*Properties.*—Hippuric acid crystallizes in semi-transparent, milk-white, long, four-sided rhombic prisms or columns, or in needles on rapid crystallization. They dissolve in 600 parts cold water, but more easily in hot water. It is soluble in alcohol, but with difficulty in ether. It is insoluble in petroleum ether, benzol, and carbon disulphide. Hippuric acid is readily soluble (about 12 times) in acetic ether. It combines with bases, forming salts which are soluble in water or alcohol. The combinations with the alkalies and the alkaline earths are readily soluble in alcohol, while the silver, copper, and lead salts are soluble with difficulty. On boiling with caustic alkalies, mineral acids, or by continued heating with water at 170°–180° C. it splits into benzoic acid and glycoll.

### Allantoin,



*Preparation.*—1. Evaporate the amniotic fluid of a cow to one sixth ( $\frac{1}{6}$ ) its volume, or calf urine to syrupy consistency,

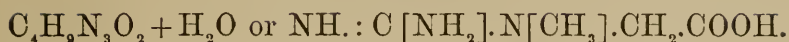
and allow it to stand in a cold place for several days, when allantoin and magnesium phosphate crystallize out, with gelatinous magnesium urate. Now dilute with water, stir, and decant the liquid from the allantoin and magnesium-phosphate crystals. Repeat this several times, and boil the crystals with water after having added some animal charcoal; filter while hot; faintly acidify the filtrate with HCl, which keeps the phosphates in solution, and allow to crystallize. Purify by recrystallization from water. (*Wöhler.*)

2. Make a thin paste of uric acid and water, heat nearly to boiling, and add in small quantities a thin paste of finely ground lead peroxide with water, continually stirring until the chocolate-brown color of the mixture just disappears. (Care must be taken not to have an excess of lead peroxide.) If the mass has become too thick, some water may be added during the operation; now filter while hot, and allow to cool. After crystallization the mother-liquor is concentrated by evaporation so as to obtain more allantoin. The preparation is purified by repeated crystallization from small amounts of boiling water. (*K. Hoffmann.*)

3. Treat 3 mols. uric acid, subdivided in water, with 1 mol. potassium permanganate, taking care that the mixture does not get warm. Filter quickly, supersaturate with acetic acid and allow to stand 24 hours, when crystals of allantoin will be formed. (*K. Hoffmann.*)

*Properties.*—Allantoin is a colorless substance, often crystallizing in prisms, or, when prepared from calf urine, as small, thin columns. It is soluble in 160 parts cold (20° C.) and 30 parts boiling water. It is insoluble in cold absolute alcohol and ether. Allantoin combines with acids and bases, forming salts. With silver oxide and mercuric oxide it forms important combinations. Allantoin reduces Fehling's solution on continued boiling.

## Creatin,



Syn. METHYL GUANIDIN ACETIC ACID.

*Preparation*.—1. Beef extract (Liebig's) is treated with water and the albumin removed as far as possible by heating to boiling, and filtering. The filtrate is precipitated by the careful addition of basic lead acetate, taking care not to add too great an excess of the precipitant. Filter, remove the excess of lead acetate in the filtrate by  $\text{H}_2\text{S}$ , and evaporate the filtrate to a small volume at moderate temperature. Allow the solution to stand one week in a cool place, filter off the separated crystals, wash with 88% alcohol, and purify when necessary by recrystallization. The crystals may be decolorized by dissolving them in hot water to which animal charcoal has been added, filtering, and allowing the filtrate to crystallize.

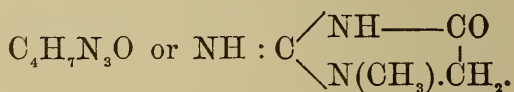
2. Finely chopped meat is mixed with one half its weight of water, pressed, and the residue again extracted with the same amount of water. This extract is freed from albumin by heating to boiling and filtering, and adding a solution of baryta water to the filtrate as long as a precipitate forms. Filter off the precipitated phosphates, remove the excess of baryta by means of carbon dioxide, filter, and evaporate the filtrate to one twentieth of its volume on the water-bath. The syrup is allowed to stand several days in a cool place when crystals will form. If a skin should form on the surface this should be removed.

3. Chopped meat is treated with ether and allowed to stand for a few days, when a strongly acid fluid separates out. This is colored red, due to myohæmatin. This watery fluid is separated and evaporated, when crystals of creatin will separate out, which may be purified by recrystallization as above. (*MacMunn*.)

*Properties*.—Creatin crystallizes in hard, colorless, shining,

monoclinic prisms containing 1 mol. water of crystallization, which it loses when heated to 100° C. It dissolves in 74 parts water at the ordinary temperature, and in 9400 parts absolute alcohol. It is insoluble in ether. Its watery solution is neutral in reaction, and has a bitter taste. Creatin forms crystalline compounds with mineral acids and with mercury. When heated with dilute mineral acids it is converted into creatinin. It forms compounds with certain metallic solutions.

### Creatinin,



### METHYL GUANIDIN HYDANTOIN.

*Preparation.*—1. Evaporate several litres of urine to one third or one quarter its volume, allow to cool, and decant liquid from the precipitated salts, now precipitate with lead acetate and filter. The excess of lead in the filtrate is removed by  $\text{H}_2\text{S}$  or soda solution; filter again, nearly neutralize with soda solution or acetic acid, heat to drive off  $\text{H}_2\text{S}$ , and add a concentrated solution of mercuric chloride, which causes a precipitate. Filter, suspend the precipitate in water and decompose it with a current of  $\text{H}_2\text{S}$ , and again filter. Decolorize the filtrate with animal charcoal and evaporate to dryness. The mass is recrystallized several times from strong alcohol. The creatinin may be easily obtained pure from the creatinin hydrochloride by treating with lead oxyhydrate and then crystallizing.

2. One-half to one litre of urine is treated with baryta mixture (1 vol. saturated  $\text{Ba}(\text{NO}_3)_2$  solution and 2 vols. saturated baryta-water) until no further precipitate is formed, filtered, and the filtrate evaporated on the water-bath to a thin syrup. This is mixed with an equal volume alcohol and allowed to stand in a cool place for 24 hours; the salts which separate out are removed by filtration and the filtrate treated



with 1 to 2 c.c. concentrated alcoholic solution of acid-free zinc chloride. After a few days yellow crystals of creatinin zinc chloride separate out; these are filtered off, washed with alcohol, dissolved in hot water and decomposed by boiling for about half an hour with an excess of freshly precipitated lead oxide or lead carbonate. Filter while hot, decolorize the filtrate by boiling with animal charcoal, filter again, evaporate to dryness, and extract the residue by strong alcohol in the cold, which removes the creatinin. Creatin remains behind undissolved.

3. Precipitate  $\frac{1}{2}$  to 1 litre of urine with phospho-tungstic acid and hydrochloric acid, filter, and treat the precipitate with caustic baryta. Filter, and remove the excess of baryta by means of carbon dioxide, and filter again. Evaporate the filtrate to dryness, extract the residue with strong alcohol and evaporate the alcohol, when the creatinin will be obtained impure. It may be purified as above described.

*Properties.*—Creatinin crystallizes in long, colorless, highly refractive, monoclinic prisms, which do not become white with loss of water when heated to  $100^{\circ}$  C. It dissolves in 11.5 parts cold water and 100 parts cold alcohol, though it is more soluble in hot liquids. It is nearly insoluble in ether. Creatinin combines with HCl and certain metallic salts, forming crystalline compounds. Creatinin acts as a reducing agent, reducing Fehling's and Trommer's solutions; also reducing mercuric oxide to metallic mercury, and yielding oxalic acid and methyl guanidin.

### Xanthin Bases.

XANTHIN,	$C_5H_4N_4O_2$ .
HYPOXANTHIN,	$C_5H_4N_4O$ .
ADENIN,	$C_5H_5N_5$ .
GUANIN,	$C_5H_5N_5O$ .

*Preparation.*—250 grms. finely chopped beef liver is placed



in a large flask, provided with a glass stopper, and treated with 2.5 litres chloroform water (2.5 litres tap water with 12.5 c.c. chloroform, and shaken until the latter is dissolved); add 2.5 c.c. chloroform, and place this mixture in an incubator and allow to digest at 40° C. for two to three days, continually shaking. After digestion transfer to a large dish and heat to boiling, and continue boiling after acidifying with acetic acid until the albumin is precipitated. Now filter, and evaporate the filtrate to 800–1000 c.c. Make alkaline with ammonia, filter off the precipitate formed, and completely precipitate with a 3% silver-nitrate solution. The gelatinous precipitate formed consists of the silver combination of the xanthin bases. Care must be taken not to have a silver-chloride precipitate form, otherwise more ammonia must be added. Filter off the precipitate, wash well, place the precipitate in a flask while still moist, and dissolve by the aid of heat in nitric acid of sp. gr. 1.1 (equal volumes nitric acid and water), adding a little urea to prevent the formation of nitrous acid. The solution should be nearly clear. Filter while hot and allow to stand for 24 hours. A precipitate of guanin, adenin, and hypoxanthin silver nitrate will have formed, while xanthin silver nitrate remains in solution. Filter and wash with dilute nitric acid.

Add ammonia to the filtrate until alkaline, when a precipitate of xanthin silver nitrate will form as a brownish or reddish flocculent precipitate. Filter, wash, suspend in water with a few drops ammonia; heat, add a few drops ammonium sulphide, stir, filter off the silver sulphide, and evaporate the filtrate, when xanthin will be obtained. (The silver compound may also be decomposed by HCl, but then on evaporation xanthin hydrochloride is produced.) The xanthin obtained as above is never quite pure.

The residue of hypoxanthin, guanin, and adenin silver compounds is suspended in water and decomposed with a current of  $H_2S$ , filtered, and the filtrate evaporated to a

small volume. This is made alkaline with ammonia and allowed to stand, when a deposit of guanin will occur, while the hypoxanthin and adenin (besides ammonium nitrate) remain in solution. Filter off the guanin and wash with water. The filtrate is evaporated to dryness on the water-bath and the residue extracted with small amounts of water to remove the ammonium nitrate, leaving the insoluble hypoxanthin and adenin.

*Properties.*—Xanthin is amorphous, or forms masses of crystalline leaves. It is soluble with difficulty in water (in 14,151 to 14,600 parts water at  $+16^{\circ}\text{C.}$  and in 1300 to 1500 parts at  $100^{\circ}\text{C.}$ ). It is insoluble in alcohol or ether, but is dissolved by alkalies or acids. Xanthin forms a crystalline, difficultly soluble combination with  $\text{HCl}$ . With very little caustic soda, xanthin gives a crystalline combination which is readily soluble in more alkali. When dissolved in ammonia, xanthin gives a gelatinous precipitate of xanthin silver, with silver nitrate. This body is soluble in nitric acid. A watery solution of xanthin is precipitated on boiling with copper acetate. Xanthin is precipitated at the ordinary temperatures by mercuric chloride and also by ammoniacal basic lead acetate.

Hypoxanthin occurs as colorless, crystalline needles, which are soluble in 300 parts cold and 78 parts boiling water. It is nearly insoluble in alcohol, but is soluble in acids or alkalies. The combination with  $\text{HCl}$  is crystalline, but is more soluble than the corresponding combination with xanthin. The silver combination with hypoxanthin dissolves with great difficulty in boiling nitric acid. Hypoxanthin picrate is difficultly soluble, but if a boiling solution is treated with a neutral or faintly acid solution of silver nitrate, the hypoxanthin is quantitatively precipitated as a combination, having the formula  $\text{C}_5\text{H}_3\text{AgN}_4\text{O.C}_6\text{H}_2(\text{NO}_2)_3\text{OH}$ .

Adenin crystallizes with 3 mol. water of crystallization in long needles, which become opalescent when heated or ex-

posed to the air. When the crystals are slowly heated with insufficient water from a solution they become suddenly opalescent at  $+53^{\circ}\text{C}$ . It is soluble in 1086 parts cold water, and much more readily soluble in hot water. Adenin is insoluble in ether, but somewhat soluble in hot alcohol. It is readily soluble in acids or alkalies. It is more soluble in ammonia than guanin, but less soluble than hypoxanthin. The silver combination of adenin is difficultly soluble in warm nitric acid, and deposits crystals of adenin silver nitrate on cooling. It also gives a combination with picric acid which is soluble with difficulty.

Guanin forms an amorphous, colorless powder, but crystallizes in very small crystals from a solution in concentrated ammonia if allowed to spontaneously evaporate. It is insoluble in water, alcohol, or ether, readily soluble in mineral acids or alkalies, but with difficulty in ammonia. The HCl salt of guanin crystallizes readily, and on account of its action on polarized light may be made use of in the identification of guanin. Picric or metaphosphoric acids precipitate even very dilute solutions of guanin. The silver combination is very difficultly soluble in boiling nitric acid, and deposits crystals on cooling.

### Urobilin.

*Preparation.*—1. Several litres of urine are acidified with  $\text{H}_2\text{SO}_4$ , using 2 grms. for every litre; then saturated with ammonium sulphate, filtered, and washed with a saturated solution of ammonium sulphate faintly acidified with sulphuric acid. The residue of urobilin is dissolved in alcohol, which requires some time. The filtered alcoholic solution is mixed with chloroform and a volume of water equal to the alcoholic solution, well shaken together, and allowed to stand until the chloroform has settled. The chloroform is removed by means of a separatory funnel, and washed with twice its volume of water. The clear chloroform solution is now filtered through

a dry filter into a distilling flask and the chloroform distilled off. The residue in the flask is washed with ether, which only dissolves very little of the urobilin. This residue is again dissolved in chloroform, filtered, and allowed to evaporate at a very moderate temperature. (*Méhu.*)

2. Precipitate the urine with basic lead acetate, wash the precipitate with water, dry at the ordinary temperature, and then boil it with alcohol, acidified with sulphuric acid. The filtered alcoholic solution is diluted with water, now saturated with ammonia, and then treated with a zinc-chloride solution. This new precipitate is washed free from chlorine with water, boiled with alcohol, dried, dissolved in ammonia, and this solution precipitated with sugar of lead. This precipitate, which is washed with water and boiled with alcohol, is decomposed by alcohol containing sulphuric acid; the filtered alcoholic solution is mixed with  $\frac{1}{2}$  vol. chloroform, diluted with water and shaken repeatedly, but not too energetically. The urobilin is taken up by the chloroform. This last is washed once or twice with a little water and then filtered, leaving the urobilin on evaporation of the chloroform. It may be further purified by treating with ether.

3. The urine is treated with ammonia in not too great excess, filtered, and the filtrate precipitated with a concentrated watery or alcoholic solution of zinc chloride. If the filtrate from this precipitate is still deeply colored, precipitate by adding more ammonia and filtering. The voluminous, generally red or reddish-brown precipitate is washed free from chlorine by first using cold and then hot water, now boiling with alcohol, and drying at a low temperature. The mass is powdered and dissolved in ammonia, leaving a small residue, and this ammoniacal solution is precipitated with sugar of lead, and the red precipitate filtered off and washed with water until the coloring matter commences to go into solution. The precipitate is now digested with alcohol acidified with sulphuric acid, filtered, and the filtrate repeatedly



shaken with its  $\frac{1}{2}$  vol. chloroform and considerable water. The chloroform is separated and washed once or twice with water and the chloroform now distilled off. The residue, consisting of impure urobilin, is washed with ether, which removes considerable quantities of a red coloring matter, and leaves the urobilin as brown amorphous masses.

*Properties.*—Urobilin, according to *Jaffé*, is amorphous, red, dingy red or reddish yellow, according to the method of preparation. It is readily soluble in alcohol, amyl alcohol, acetic ether, and chloroform, but less readily in ether or water. It is soluble in alkalies, and is incompletely precipitated from the alkaline solution by the addition of acid. Its alkaline solutions give insoluble combinations with salts of the heavy metals, such as zinc and lead. A neutral solution of urobilin gives a green fluorescence, and the acid solution shows a faint absorption-band between  $b$  and  $F'$ , which borders on  $F'$ , or in greater concentration extends over  $F'$ . The alkaline solutions show a darker or more sharply defined absorption-band, almost midway between  $b$  and  $F'$ .

### Glycuronic Acid,



*Preparation.*—Purée or Indian yellow is rubbed fine with water in a mortar, forming a thick mass. This is acidified with hydrochloric acid, filtered, and thoroughly washed with water. The euxanthic acid thus obtained is dissolved in hot alcohol, filtered, and allowed to stand to crystallize as beautiful yellow needles. This crystallization is repeated again so as to obtain pure euxanthic acid. One part of the euxanthic acid is treated with 150 parts water and heated, in a Papin's digester supplied with a thermometer, first to boiling, allowing the cover of the digester to be open. After closing the cover heat for an hour, keeping the temperature at  $120^\circ$ – $125^\circ$  C. On cooling the liquid is filtered, leaving the crystals of unde-



composed euxanthic acid and euxanthon on the filter. These are again treated with water as above. The filtrate is evaporated in a flat dish, care being taken not to have too high a temperature. When at a syrupy consistency it is allowed to stand, when crystals of glycuronic acid anhydride are obtained. (*Thierfelder.*)

*Properties.*—Glycuronic acid is not crystalline, but is obtained only as a syrup. It dissolves in alcohol, and is easily soluble in water. It forms crystalline salts with potassium and sodium. The neutral lead salt is soluble in water, but the basic salt is, on the contrary, insoluble. The acid is dextro-rotatory ( $\alpha$ ) $D = +19.4$ , and reduces alkaline solutions of copper, silver, and bismuth salts. It gives a crystalline combination with phenyl hydrazine, melting at  $114^{\circ}$ – $115^{\circ}$  C.



LIST OF  
IMPORTANT TESTS  
ARRANGED IN ALPHABETICAL ORDER.



## LIST OF IMPORTANT TESTS ARRANGED IN ALPHABETICAL ORDER.

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ACETONE. See *Chautard, Gunning, Lieben, Legal, Le Nobel, Malerba, Penzoldt, Reynolds.*

ACETO-ACETIC ACID. See *Gerhardt.*

ADAMKIEWICZ'S Reaction (*Proteids*).—Add the proteid to a mixture of 1 vol. concentrated sulphuric acid and 2 vols. glacial acetic acid. A reddish-violet color is obtained slowly at the ordinary temperature, but more quickly on heating. The liquid has also a feeble fluorescence, and gives an absorption band between the lines *b* and *F* in the solar spectrum.

ALBUMIN. See *Axenfeld, Boedeker, Cohen, Furbringer, Heller, Heynsius, Hindenlang, Johnson, McWilliam, Méhu, Oliver, Pavy, Reoch, Roberts, Rees, Raabe, Spiegler, Tanret, Zouchlos.*

ALLEN'S Test (*Phenol*).—Add to one to two drops of the liquid to be tested a few drops of hydrochloric acid and then 1 drop of nitric acid. Cherry-red color is produced, which is intensified by gentle warming. Alcohol does not interfere with the reaction. On supersaturating with caustic soda the red liquid becomes dark brown,



ALMÉN'S Reaction (*Blood*).—Mix in a test-tube equal volumes of tincture of guaiacum and old turpentine which has become strongly ozonized by the action of air under the influence of light. Allow the liquid to be tested to flow down gently on the surface of this mixture. If blood or blood-coloring matters are present, a bluish-green and then a beautiful blue ring appears where the two liquids come together, and if shaken the liquid becomes more or less blue. Pus gives a blue color with this mixture, but in this case the tincture of guaiacum alone, without turpentine, is colored blue.

ALMÉN'S Test (*Glucose*).—Heat liquid with a solution of bismuth subnitrate dissolved in caustic soda and Rochelle salts. If glucose is present, the liquid becomes dark, cloudy, dark brown, or nearly black, and non-transparent. After a time a black deposit appears.

ANDREASCH'S Reaction (*Cystein*).—Treat the hydrochloric acid solution with a few drops dilute ferric chloride solution and then ammonia. The liquid will become beautifully red, darkening on shaking with air.

AXENFELD'S Test (*Albumin in Urine*).—Acidify with formic acid and add, drop by drop, a 0.1 per cent gold chloride solution and warm. The solution becomes first red, then purple red, and, on the further addition of gold chloride, blue, and lastly, a blue precipitate is produced. The red coloration is characteristic of albumin, while the blue and violet may be produced by other bodies, such as glucose, glycogen, starch, leucin, tyrosin, uric acid, urea, creatinin, etc.

BAEYER'S Reaction (*Glucose*).—On boiling a glucose solution with ortho-nitrophenyl propionic acid and sodium carbonate indigo is formed. With an excess of glucose this blue is converted into indigo white.

BAEYER'S Reaction (*Indol*).—A watery solution of indol gives with fuming nitric acid a red liquid and then a red precipitate of nitroso-indol nitrate,  $C_{16}H_{13}(NO)N_{21}HNO_3$ . It is better to first add two or three drops of nitric acid and then a 2% solution of potassium nitrite, drop by drop. (*Sal-kowski*.)

BARFOED'S Reagent (*Dextrose*).—Dissolve 1 part copper acetate in 15 parts water; to 200 c.c. of this solution add 5 c.c. of acetic acid containing 38 per cent of glacial acetic acid. On heating this reagent with a dextrose solution a reduction of copper suboxide is produced, but not when heated with lactose or maltose.

BAUMANN'S Reaction (*Dextrose*).—If a watery solution of grape sugar is treated with benzoyl chloride and an excess of caustic soda, and shaken until the odor of benzoyl chloride has disappeared, a precipitate of benzoic acid ester of dextrose will be produced, which is insoluble in water or alkalies.

BAUMANN and GOLDMAN'S Test (*Cystin*).—Shake the solution of cystin in caustic soda with benzoyl-chloride; a voluminous precipitate of benzoyl cystin is produced. The sodium salt precipitates as silky plates, which are readily soluble in water, but nearly insoluble in an excess of caustic soda.

BERTHELOT'S Test (*Phenol*).—On adding sodium hypochlorite to an ammoniacal solution of phenol a beautiful blue coloration is produced.

BILE ACIDS. See *Drechsel*, *Mylius*, *Pettenkofer*, *Strassburg*, *Udransky*.

BILE PIGMENTS. See *Capranica*, *Dragendorff*, *Dumontpallier*, *Fleischl*, *Gmelin*, *Huppert*, *Jolles*, *Le Nobel*, *Maréchal*, *Rosenbach*, *Stokvis*, *Smith*, *Trousseau*, *Ultzmann*, *Vitali*.

BILIRUBIN. See *Ehrlich*.

BIURET Reaction (*Proteids*). See PIOTROWSKI'S Reaction.

BIURET Reaction (*Urea*).—Heat urea in a dry test tube until completely molten ; continue the heat for some time. When cold, dissolve in water, add abundant caustic soda and a dilute solution of copper sulphate, drop by drop. The solution becomes first pink, then reddish violet, and lastly bluish violet, the more copper sulphate solution is added.

BLOOD. See *Almén, Hoppe-Seyler, Hayem, Kobert, Lenden-dorff, Pacini, Rubner, Salkowski, Struve, Wetzel, v. Zaleski*.

BOAS'S Test (*HCl in Contents of Stomach*).—Dissolve 5 grms. pure resorcin and 5 grms. white sugar in 100 grms. dilute alcohol. A few drops of this reagent are spread out in a thin layer upon a porcelain dish, and then gently heated. On allowing a drop of the filtrate from the stomach to flow across it, or a glass rod dipped in the solution touched to it, a deep scarlet streak is developed. If the solution is very dilute, no change is observed until the solution evaporates entirely to dryness.

BOEDEKER'S Reaction (*Albumin*).—Acidify the liquid with acetic acid and add a solution of potassium ferrocyanide, drop by drop. White precipitate of albumin will be formed.

BÖTTGER'S Test (*Dextrose*).—Make the liquid alkaline with carbonate of soda or potash, add some solid bismuth subnitrate and boil. The presence of dextrose is shown by the darkening of the bismuth salt or a black precipitate.

BRAUN'S Reaction (*Glucose*).—Warm the glucose solution with caustic soda or potash until it is yellow ; now drop into this a dilute solution of picric acid, and heat to boiling. A

deep red coloration will be the result. Creatinin gives this same reaction even in the cold, also acetone, though faintly.

BRÜCKE's Reagent (*Proteids*).—Saturate a boiling 10 per cent solution of potassium iodide with freshly precipitated mercuric iodide; on cooling this is filtered, and the filtrate employed with hydrochloric acid as a precipitant for the proteids.

CAPRANICA's Reactions (*Guanin*).—1. A warm solution of guanin hydrochloride with a cold saturated solution of picric acid gives a yellow precipitate, consisting of silky needles.

2. With a concentrated solution of potassium chromate guanin solutions give an orange-red crystalline precipitate, very insoluble in water.

3. On the addition of a concentrated solution of potassium ferricyanide to a guanin solution a prismatic, yellowish-brown precipitate is formed.

CAPRANICA's Reaction (*Bile Pigments*).—Shake the solution with chloroform containing some bromine; it becomes first green, indigo blue, violet, yellowish red, and lastly colorless. If the green or blue solution is shaken with HCl, the color is taken up by the acid.

CELLULOSE. See *Schulze*, *Schweitzer*.

CHAUTARD's Test (*Acetone*).—Pass sulphurous acid through a solution of 0.25 grms. fuchsin in 500 c.c. water until the solution is yellow in color. Add to a portion of this the liquid to be tested for acetone. If present, the liquid will be colored violet.

CHOLESTERIN. See *Liebermann*, and *Burchard*, *Obermüller*, *Salkowski*, *Schiff*, *Schulze*, *Zwenger*.

*Cholesterin*.—1. The crystal is treated with a mixture of 5 parts sulphuric acid and 1 part water, when colored rings are produced, first a bright carmine red and then violet.

2. On the addition of a little iodine solution to the above the crystals will be colored variously—blue, red, green, violet.

3. If a trace of cholesterin is gradually heated to dryness with a few drops of nitric acid, a yellow spot is produced, which turns red on the addition of ammonia. This red color is not changed by the addition of caustic soda, thus differing from the murexid test for uric acid.

CIAMICIAN and MAGNANINI'S Test (*Skatol*).—On warming skatol with sulphuric acid a beautiful purple-red coloration is produced.

COHEN'S Test (*Albumin*).—Add a solution of potassium iodide and potassium bismuthic iodide to the acid solution of albumin. Precipitation of the albumin occurs. Alkaloids are also precipitated.

CREATININ. See *Jaffé, Kerner, v. Maschke, Salkowski, Weyl*.

CRISMER'S Test (*Glucose*).—A solution of 1 part safranine in 1000 parts water is decolorized or yields a pale yellow color when heated to boiling with an alkaline solution of glucose. Safranine solution is not decolorized when heated in alkaline solution with uric acid, creatin, or creatinin.

CYSTEIN. See *Andreasch*.

CYSTIN. See *Baumann and Goldmann, Liebig, Müller*.

DAVY'S Test (*Phenol*).—Add 3–4 drops molybdic-sulphuric acid (a solution of 1 part molybdic acid in 10 or more parts conc. sulphuric acid) to 1–2 drops of the phenol solution. A pale yellow or yellowish-brown coloration is the result, which



passes to a chestnut or reddish brown and then to a beautiful purple. Gentle heat facilitates the reaction.

DENIGÈS'S Test (*Uric Acid*).—If uric acid is converted into alloxan by the careful action of nitric acid, and the excess of nitric acid expelled by gentle warming and then treated with a few drops sulphuric acid and also a few drops commercial benzol (containing thiophen), a blue coloration is produced.

DEXTROSE. See *Glucose*.

DIETRICH'S Reaction (*Uric Acid*).—Add a solution of sodium hypochlorite or hypobromite to the uric acid solution, when a red coloration is produced. This coloration disappears on adding caustic alkali.

DONNÉ'S Test (*Pus*).—Stir a small piece of caustic potash with the mass to be tested. If pus is present, the mass is converted into a slimy tough material.

DRAGENDORFF'S Test (*Bile Pigments*).—Place a few drops of the urine on an unglazed porcelain surface and when it has been absorbed add a drop or two of nitric acid. Several rings of color will be produced if bile is present, chief amongst these rings being the green ring, which is characteristic of bile pigments.

DRECHSEL'S Test (*Bile Acids*).—Treat the substance with a little cane sugar and a few drops of a mixture of 5 vols. syrupy phosphoric acid and 1 vol. water. Warm on water-bath, when a beautiful red coloration is produced if bile acids are present.

DUMONT-PALLIER'S Test (*Bile Pigments*). See SMITH'S Test.

EIJKMAN'S Test (*Phenol*).—Mix the phenol solution with a few drops of an alcoholic solution of nitrous acid ethyl ether

and an equal volume concentrated sulphuric acid. A red coloration is the result.

EISELT'S Reaction (*Melanin in Urine*).—Urine containing melanin becomes dark-colored with oxidizing reagents, such as concentrated nitric acid, potassium dichromate, and sulphuric acid, as well as with free sulphuric acid.

EHRlich's Reaction (*Bilirubin*).—To a solution of bilirubin in chloroform add an equal volume or twice its volume of a solution of sulpho-diazobenzol (1 grm. sulphanilic acid, 15 c.c. of hydrochloric acid, and 0.1 grm. sodium nitrite, diluted to 1 litre with water). Then add as much alcohol as is needed to render the solution clear. The liquid, which is of a yellow color at first, assumes a beautiful red tint. On adding HCl, drop by drop, the color changes first to violet and then to an intense blue. On now carefully pouring into the test-tube a solution of potassium or sodium hydrate three zones of color are visible: near the alkaline solution, where the reaction begins, the color is green; at the surface, where the reaction is still acid, the original blue tint persists; whilst intermediate between these two zones is a red, neutral zone.

EWALD'S Test (*Hydrochloric Acid in contents of stomach*).—Dilute 2 c.c. of a 10% solution of potassium sulpho-cyanide and 0.5 c.c. of a neutral solution of iron acetate to 10 c.c. with water. Place a few drops of this ruby-red solution in a porcelain dish and allow 1–2 drops of the liquid to be tested to flow gently thereon. In the presence of HCl a faint violet cloud is observed where the two liquids come in contact with each other. On mixing the color becomes mahogany brown. Peptones or salts do not interfere with this reaction.

FEHLING'S Reagent (*Glucose*).—1. Dissolve 34.65 grammes pure copper sulphate in 1000 c.c. water.

2. Dissolve 173 grammes Rochelle salts in 350 c.c. water, adding 600 c.c. of a caustic soda solution of a specific gravity of 1.12, and dilute to 1000 c.c. with water. For use mix equal parts of the above solutions and dilute with an equal volume of water.

Fehling's Solution.

10 c.c. = 0.0500 gramme dextrose, lævulose, or invert sugar.

10 c.c. = 0.0475 gramme cane-sugar (after inversion).

10 c.c. = 0.07143 gramme milk-sugar (lactose).

10 c.c. = 0.0807 gramme malt-sugar (maltose).

FLEISCHL'S Reaction (*Bile Pigments*).—Treat the urine with a concentrated solution of sodium nitrate and add concentrated sulphuric acid by means of a pipette. This latter sinks to the bottom of the test-tube and produces the coloration, as in *Gmelin's* test.

FRÖHDE'S Reaction (*Proteids*).—On heating a solid proteid with sulphuric acid containing molybdic acid a beautiful dark blue color is produced.

FROHN'S Reagent.—Treat 1.5 grammes freshly precipitated bismuth subnitrate with 20 c.c. water, heat to boiling, and then add 7 grammes potassium iodide and 10 c.c. hydrochloric acid.

FURBRINGER'S Reagent (*Albumin*).—Gelatin capsules containing the double salt of mercuric chloride and sodium chloride with citric acid.

FURFUROL Reaction (*Proteids*).—On heating proteids with sulphuric acid furfural is produced, which may be detected by various means (see MOLISCH, SCHULTZE.)

GALLOIS'S Test (*Inosit*).—Evaporate the inosit solution to incipient dryness and moisten the residue with a little mercuric nitrate solution, when a yellowish residue is obtained on

drying. This yellow color becomes beautifully red on strongly heating, but disappears on cooling, but reappears on gently heating again.

**GENTELE'S Test (*Glucose*).**—If a glucose solution is added to a solution of potassium ferricyanide made alkaline with caustic soda or potash, it is decolorized with the formation of potassium ferrocyanide on gently warming. Uric acid also gives this same reaction even in the cold.

**GERHARDT'S Reaction (*Aceto-acetic Acid*).**—Aceto-acetic acid gives a wine-red coloration with a dilute, not too acid, ferric chloride solution. In testing urine treat 1–15 c.c. with ferric chloride as long as it gives a precipitate, filter the precipitate of ferric phosphate, and add some more ferric chloride to the filtrate. In the presence of the acid a claret-red color is produced. The urine may also be acidified with sulphuric acid and shaken with ether (which takes up the acid). Now shake the removed ether with a very dilute watery solution of ferric chloride and the watery layer becomes violet red or claret red. The color disappears on warming.

**GERHARDT'S Test (*Urobilin*).**—Extract the urobilin from the solution by shaking with chloroform. Treat this chloroform extract with iodine solution and then a solution of caustic potash, when a beautiful green fluorescence is the result.

**GERRARD'S Test (*Glucose*).**—Add a 5% solution of potassium cyanide to Fehling's solution until the blue color just begins to disappear. On heating this solution to boiling with a glucose solution no precipitation of cuprous oxide is produced, but the solution will be decolorized more or less.

**GLOBULIN.** See *Hammarsten, Pohl*.

**GLUCOSE.** See *Almén, Barfoed, Böttger, Baumann, Baeyer, Braun, Crismer, Fehling, Gentele, Gerrard, Haines, v.*

*Jaksch, Knapp, Löwenthal, Moore, Molisch, Mulder, Nylander, Penzoldt, Pavy, Roberts, Rubner, Soldani, Schiff, Trommer, Wenders.*

**GMELIN'S Test (*Bile Pigments*).**—If nitric acid containing some nitrous acid is carefully poured beneath a solution containing bile pigments, a series of colored layers are obtained at the juncture of the two liquids in the following order from above downwards: green, blue, violet, red, and reddish yellow. The green ring must never be absent, and also the reddish violet must be present at the same time, otherwise the reaction may be confused with that for lutein, which gives a blue or greenish ring. The nitric acid must not contain too much nitrous acid, for then the reaction takes place too rapidly and does not become typical. Alcohol must not be present, because it gives a play of colors with the acid.

**GRIESS'S Reagent (*Nitrous Acid*).**—A solution of metadiamido-benzol (melting at 63° C.) gives an intense yellow coloration with dilute solutions containing nitrous acid which have been acidified with a few drops sulphuric acid.

**GRIGG'S Test (*Proteids*).**—A solution of meta-phosphoric acid gives a precipitate with all proteids with the exception of the peptones.

**GUANIN.** See *Capranica*.

**GUNNING'S Test (*Acetone*).**—Add an alcoholic solution of iodine to the liquid to be tested and then ammonia. On standing a precipitate of iodoform and a black precipitate of iodide of nitrogen is formed, but this latter gradually disappears on standing, leaving the iodoform visible.

**GUNZBURG'S Reagent (*Hydrochloric Acid*).**—Dissolve 2 grms. phloroglucin and 1 grm. vanillin in 100 c.c. alcohol. In testing for the presence of free HCl add an equal amount of



the above reagent to the liquid to be tested in a porcelain dish and evaporate the mixture on the water-bath. In the presence of HCl a delicate rose-red coloration is observed in the residue in the porcelain dish.

HÆMIN. See *Teichmann*.

HÆMOGLOBIN. See *Kobert*.

HAINES'S Solution (*Glucose*).—Dissolve 30 grains pure copper sulphate in  $\frac{1}{2}$  ounce distilled water and add  $\frac{1}{2}$  ounce pure glycerin, mix thoroughly and add 5 ounces liquor of potassæ.

HAMMARSTEN'S Test (*Globulin*).—Add powdered magnesium sulphate to the neutral solution until no more of the salt dissolves. The globulin will be thus precipitated and separated by filtration and washed with a saturated solution of magnesium sulphate.

HAYEM'S Solution (*Blood*).—This solution is prepared by dissolving 1 gram. sodium chloride, 5 grms. sodium sulphate, 0.5 gram. mercuric chloride, in 200 c.c. distilled water. It is used in the microscopical examination of the form elements of the blood.

HELLER'S Test (*Albumin*).—Float the liquid to be tested on the surface of nitric acid. The presence of albumin is shown by a well-defined white ring between the two liquids. With this test even 0.02 p.m. albumin may be detected without difficulty.

HERZBERG'S Reagent (*Free Inorganic Acids*). — Paper moistened with a solution of Congo red and dried turns bluish black or blue when moistened with hydrochloric acid. The delicacy of this reaction is diminished by the presence of proteids or salts in large quantities.

**HEYNSIUS'S Test (*Albumin*).—**Strongly acidify the solution with acetic acid and add a few cubic centimetres of a saturated solution of sodium chloride and boil. In the presence of albumin a flocculent precipitate is produced.

**HINDENLANG'S Test (*Albumin*).—**Add solid meta-phosphoric acid to the liquid to be tested, when a cloudiness or precipitate is formed if albumin is present.

**HIPPURIC ACID.** See *Lücke*.

**HOFMANN'S Test (*Tyrosin*).—**Add a few drops *Millon's* reagent to the solution to be tested and boil for a time. In the presence of tyrosin the liquid becomes a beautiful red and then yields a red precipitate. The test may also be applied by first adding mercuric nitrate and boiling, and after this adding nitric acid containing some nitrous acid.

**HOFMEISTER'S Test (*Peptones*).—**A solution entirely free from albumin gives a precipitate with an acetic acid solution of phospho-tungstic acid. The phospho-tungstic acid may be prepared by dissolving commercial sodium tungstate in hot water and adding phosphoric acid until acid in reaction. This liquid is strongly acidified with hydrochloric acid after cooling and filtered after 24 hours.

**HOFMEISTER'S Test (*Leucin*).—**On warming a solution of leucin with mercurous nitrate a deposit of metallic mercury is formed.

**HOPPE SEYLER'S Test (*Carbon Monoxide in Blood*).—**Treat the blood with double its volume of caustic soda solution of 1.3 sp. gr. Ordinary blood is converted into a dingy brownish mass, which when spread out on porcelain is brown, with a shade of green. Carbon monoxide blood gives under the same conditions a red mass, which if spread out on porcelain shows a beautiful red color.

HOPPE SEYLER'S Test (*Xanthin*).—Add some chloride of lime to some caustic soda in a porcelain dish and add the xanthin to this mixture; at first a dark green and then quickly a brownish halo forms around the xanthin-grains and then disappears.

HUPPERT'S Reaction (*Bile Pigments*).—Treat the solution with milk of lime or with a solution of calcium chloride and then precipitate with ammonia. This precipitate, containing bilirubin calcium, is filtered, washed with water, transferred while moist to a test-tube and treated with alcohol which has been acidified with sulphuric acid, and heated to boiling for some time, when the liquid becomes emerald green or bluish green in color.

HYDROCHLORIC ACID IN CONTENTS OF STOMACH. See *Boas, Ewald, Gunzburg, Herzberg, v. Jaksch, Mohr, Maly, Rabuteau, Reoch, Szabo, Uffelmann, v. Velden, Witz.*

HYDROGEN PEROXIDE. See *Wurster.*

HYPOXANTHIN. See *Kossel.*

INDICAN. See *Jaffé, MacMunn, Obermeyer, Weber.*

INDIGO RED. See *Rosenbach, Rosin.*

INDOL. See *Baeyer, Nencki, Salkowski.*

INOSIT. See *Gallois, Scherer, Seidel.*

JACQUEMIN'S Test (*Phenol*).—Treat the solution with an equal volume of anilin and then a solution of sodium hypochlorite, when a blue coloration is the result. Acids turn the liquid red and alkalies turn it blue again.

JAFFÉ'S Test (*Indican*).—Treat 20 c.c. of the solution to be tested with an equal volume of hydrochloric acid and add, by

means of a pipette, small amounts of a concentrated solution of chloride of lime or  $\frac{1}{2}$  per cent potassium permanganate solution, drop by drop, and after each drop shake the mixture. In the presence of indican the mixture turns blue, due to the production of indigo blue. An excess of oxidizing reagent, especially chloride of lime, interferes with the reaction, and must therefore be avoided. If 2-3 c.c. of chloroform are shaken with the blue solution, it will be colored blue by the indigo blue formed.

**JAFFÉ'S Reaction (*Creatinin*).**—Treat the solution with a rather concentrated watery solution of picric acid and a few drops of caustic potash solution. In the presence of creatinin a red coloration, lasting several hours, is produced on warming. This color changes to yellow on the addition of acid. Aceton and glucose give a similar reaction.

**V. JAKSCH'S Test (*Glucose*).**—Add in a test-tube containing 8-10 c.c. of the solution to be tested two knife-points of phenyl hydrazin hydrochloride and three knife-points of sodium acetate, and when the added salts do not dissolve on warming, add more water. The mixture is heated in boiling water for one hour. It is then poured into a beaker-glass of cold water. In the presence of glucose a precipitate consisting of groups of yellow needles of phenyl glucosazone is formed. In doubtful cases determine the melting-point of these yellow crystals to be 204-205° C.

**V. JAKSCH'S Test (*Melanin*).**—Add a few drops of a concentrated solution of ferric chloride to the liquid to be tested. In the presence of melanin it turns gray, and on the addition of more ferric chloride the precipitate, consisting of the coloring matter and the phosphates, is redissolved.

**V. JAKSCH'S Test (*HCl in Contents of Stomach*).**—Paper moistened with a saturated, watery solution of benzo-purpurin 6 B.

and dried gives with dilute solutions of HCl a beautiful violet coloration. If the paper becomes dark blue, the solution contains more than 0.4 gm. HCl in 100 c.c. of the solution.

V. JAKSCH'S Test (*Uric Acid*).—This consists in substituting chlorine or bromine water or nitrous acid for the nitric acid in the murexid test (see *Murexid* Test.) This reaction differentiates between uric acid and the xanthin bases.

JOHNSON'S Test (*Albumin*).—Float the acidified solution on a cold saturated solution of picric acid. If albumin is present, a precipitation of the albumin occurs between the two liquids.

JOLLES'S Test (*Bile Pigments in Urine*).—Place 50 c.c. of the urine in a stopper cylinder, add a few drops of 10% hydrochloric acid and an excess of a barium chloride solution with 5 c.c. chloroform, and shake for several minutes. After 10 minutes remove the chloroform and the precipitate by means of a pipette and place in a test-tube and heat on the water-bath to about 80° C. After the evaporation of the chloroform decant the liquid from the precipitate carefully and allow 3 drops concentrated sulphuric acid containing  $\frac{1}{3}$  fuming nitric acid to flow down the sides of the test-tube. In the presence of bile pigments the characteristic coloration is produced.

KERNER'S Reaction (*Creatinin*).—A solution of creatinin acidified with a mineral acid gives a crystalline precipitate with phospho-tungstic or phospho-molybdic acids, even in very dilute solutions.

KNAPP'S Solution (*Glucose*).—Dissolve 10 grms. chemically pure dry mercuric cyanide in 100 c.c. caustic soda solution of a specific gravity of 1.145 and dilute to 1 litre. On heating



a glucose solution with the above solution diluted with water a reduction of metallic mercury takes place. Ten c.c. of this solution are reduced by 0.025 grm. glucose.

ROBERT'S Test (*Hæmoglobin*).—Shake the solution with zinc powder or treat with a solution of zinc sulphate or acetate, when a precipitate of zinc hæmoglobin is formed. This precipitate when collected is colored red by alkalies.

KOSSEL'S Test (*Hypoxanthin*).—Treat the solution with zinc and hydrochloric acid and then make alkaline with caustic soda or potash. In the presence of hypoxanthin the solution becomes first ruby red and then brownish red in color.

LACTIC ACID. See *Uffelmann*.

LADENDORFF'S Test (*Blood*).—Treat the liquid with tincture of guaiacum and then with oil of eucalyptus, when the lower layer becomes blue and the upper layer violet if blood is present.

LANDOLT'S Test (*Phenol*).—On treating the solution with bromine water a white crystalline precipitate of tribromophenol ( $C_6H_2Br_3OH$ ) is produced.

LANG'S Reaction (*Taurin*).—On boiling a solution of taurin with freshly precipitated mercuric oxide a white combination occurs which appears as a precipitate.

LEGAL'S Test (*Acetone*).—Treat the acetone solution with a few drops of a freshly prepared solution of sodium nitroprusside and then with caustic potash or soda solution. The solution becomes ruby red in color, but if saturated with acetic acid the color becomes carmine or purplish red. Creatinin gives the ruby-red color, with sodium nitroprusside

and alkali, but this turns yellow, and then gradually green and blue, when saturated with acetic acid. Ammonia may be substituted for the caustic soda or potash and gives the same reaction with acetone, but no reaction with creatinin.

LE NOBEL'S Modification of LEGAL'S Test (*Acetone*).—Instead of using caustic potash or soda with the sodium nitroprusside he suggests the use of ammonia, which produces a ruby-red reaction with acetone, but not with creatinin. (See *Legal's Test*.)

LE NOBEL'S Test (*Bile Pigments*).—Treat the liquid with zinc chloride and a few drops of tincture of iodine. A dichroitic play of colors is the result.

LEUCIN. See *Hoffmeister, Scherer*.

LIEBEN'S Test (*Acetone*).—When a watery solution of acetone is treated with alkali and then a solution of iodine in potassium iodide solution and gently warmed, a yellow precipitate of iodoform is formed, which is known by its odor and by the appearance of the crystals (six-sided plates or stars) under the microscope.

LIEBERMANN-BURCHARD'S Test (*Cholesterin*).—Dissolve the substance in acetic anhydride and then add concentrated sulphuric acid, when a beautiful violet color is produced, and this passes quickly to green if cholesterin is present.

LIEBERMANN'S Test (*Proteids*).—Treat the proteid, previously washed with alcohol and ether, with concentrated fuming hydrochloric acid, when a beautiful violet-blue coloration is the result. This liquid gives an absorption-band between *E* and *b*.

LIEBIG'S Test (*Cystin*).—Boil the substance with caustic alkali containing lead oxide. If cystin is present, a precipitate of blacklead sulphide is produced.

**LÖWENTHAL'S Test (*Glucose*).**—On boiling a glucose solution with a solution of ferric chloride dissolved in tartaric acid and sodium carbonate it darkens and soon deposits a voluminous precipitate of iron oxide. This test cannot be applied to the urine, as all urines give it.

**LÜCKE'S Reaction (*Hippuric Acid*).**—Evaporate the substance to dryness with nitric acid, when an intense odor of nitro-benzol (oil of bitter almonds) is generated when the residue is heated.

**MACMUNN'S Test (*Indican in Urine*).**—Equal parts of urine and hydrochloric acid and a few drops nitric acid are boiled, cooled, and agitated with chloroform. The chloroform is colored violet and shows an absorption-band before *D*, due to indigo blue, and another after *D*, due to indigo red.

**MACWILLIAM'S Test (*Albumin*).**—Add a concentrated watery solution of salicyl sulphonic acid to the acid-reacting solution, when a cloudiness or precipitate will be formed in the presence of albumin. In the presence of peptones or albumoses the precipitate disappears on boiling, but reappears on cooling.

**MALERBA'S Test (*Acetone*).**—A solution of dimethylpara-phenylendiamine gives a red coloration with acetone, which gives an absorption spectrum very similar to oxyhæmoglobin.

**MALY'S Test (*HCl in Contents of Stomach*).**—Place liquid to be tested in a glass dish and add as much ultramarine to make it just blue. Then cover the dish with a watch-glass after having suspended a piece of lead-paper in the upper part of the dish. On warming the mixture on the water-bath after 15 minutes in the presence of HCl the blue color of the mixture has changed to brown and the lead-paper will have turned dark, due to the development of  $H_2S$ .

Zinc sulphide may be substituted for the ultramarine, using a knife-point of the powder to 20 c.c. of the filtered contents of the stomach.

MANDEL'S Test (*Proteids*).—A 5 per cent solution of chromic acid produces a precipitate with solutions of proteids. If the solution is first made acid with acetic or citric acid, the precipitate produced is flocculent and settles rapidly. It produces a marked cloudiness with 1 part albumin dissolved in 50,000 parts water. Chromic acid solution may be substituted for nitric acid in *Heller's* test, using a 10 per cent solution.

MARÉCHAL (*Bile Pigments*). See SMITH'S Test.

V. MASCHKE'S Reaction (*Creatinin*).—Dissolve the creatinin in a cold saturated solution of sodium carbonate and add a few drops of Fehling's solution. An amorphous flocculent precipitate is obtained in the cold, but better on warming to 50–60° C.

MÉHU'S Test (*Albumin*).—Shake 100 vols. of the solution with 2–3 vols. nitric acid and 10 vols. of a solution of 1 part phenol and 1 part acetic acid in 2 parts 90% alcohol. In the presence of albumin a precipitate is produced. Instead of nitric acid one half a volume of a saturated solution of sodium sulphate may be used.

MELANIN. See *Eiselt, v. Jaksch, Thormählen, Zeller*.

MICHAÏLOW'S Test (*Proteids*).—Treat the solution with ferrous sulphate, and allow concentrated sulphuric acid to flow under the solution, and then add carefully very little nitric acid. Besides a brown ring, a blood-red coloration will also be produced,

MILLON'S Reagent (*Proteids*).—Dissolve 1 part mercury in 2 parts nitric acid (sp. gr. 1.42), allow to stand some time, and then apply heat. After complete solution of the mercury add 1 vol. of this solution to 2 vols. of water. Allow to stand a few hours and decant the supernatant liquid.

This reagent gives with solutions of proteid bodies a precipitate which slowly at the ordinary temperature, but quickly at the boiling-point, turns red, depending upon the amount of albumin. Solid albuminous bodies give the same reaction. This reaction depends on the presence of the aromatic group in the proteid, and is also given by tyrosin and other benzol derivatives with a hydroxyl group in the benzol nucleus.

MOHR'S Test (*HCl in Contents of Stomach*).—A solution of iron acetate (free from alkali acetates) so diluted as to have only a light yellow color is treated with a few drops of a solution of potassium sulpho-cyanide. No change of color should be produced, but if the filtered contents of the stomach are added, and they contain free HCl, an intense red coloration is the result. This color disappears on the addition of sodium acetate.

MOLISCH'S Test (*Glucose*).—1. Treat  $\frac{1}{2}$  to 1 c.c. of the solution with 2 drops of a 15–20% alcoholic solution of a naphthol. The liquid becomes cloudy, due to the precipitation of some of the naphthol, but on the addition of 1–2 c.c. concentrated sulphuric acid a beautiful deep violet coloration is produced, which forms a violet precipitate on diluting with water.

2. Instead of employing a solution of a naphthol he also suggests the use of a 15–20% alcoholic solution of thymol, applied as above. In the presence of glucose it is colored ruby red and becomes carmine red on dilution with water.

MOORE'S Test (*Glucose*).—If a glucose solution is treated with about  $\frac{1}{4}$  of its volume of caustic soda or potash and warmed, the



solution becomes first yellow, then orange, yellowish brown, and lastly brown, depending upon the amount of glucose present. A faint odor of caramel is also observed, and this is more pronounced if the solution is acidified.

MULDER'S Test (*Glucose*).—Treat the solution with a solution of sodium carbonate and add a solution of indigo carmine. On heating in the presence of glucose the solution becomes decolorized, and turns blue again on shaking with air.

MULDER'S Test, also *Xantho-proteic* Reaction (*Proteids*).—On treating proteids with concentrated nitric acid they are colored yellow. On adding ammonia or caustic soda or potash they turn orange yellow.

MÜLLER'S Test (*Cystin*).—Dissolve the cystin by boiling with caustic potash, dilute with water when cold, and add a solution of sodium nitro-prusside, when a violet coloration is produced. This color changes rapidly to yellow.

MUREXID Test (*Uric Acid*).—Heat the powder gently on a watch-glass with a drop or two of strong nitric acid. A red residue is produced, which, when cold, turns a purple red when ammonia is added (purpurate of ammonium). When caustic soda or potash is added to this, it becomes more blue or bluish violet. Better results are obtained if the heating is done over the water-bath, and not over a naked flame.

MYLIUS'S Modification of *Pettenkofer's* Test (*Bile Acids*).—To each cubic centimetre of the alcoholic solution of bile acids add 1 drop of furfurol solution and 1 c.c. concentrated sulphuric acid, and cool when necessary, so that the test does not become too warm. A red coloration is the result, and this color does not disappear at the ordinary temperature, but becomes more bluish violet in the course of a day.

NENCKI'S Test (*Indol*).—Indol gives a pronounced red coloration with nitric acid containing nitrous acid. In concentrated solution a red precipitate may form. This reaction is not given by skatol.

NITROUS ACID or Nitrites. See *Griess*.

NYLANDER'S Test (*Glucose*).—Dissolve 4 grms. Rochelle salts in a solution of 10.33 grms. NaHO in 100 c.c. water. Add to this 2 grms. bismuth subnitrate and digest on the water-bath until as much of the bismuth salt is dissolved as possible. On heating 10 vols. of a glucose solution with 1 vol. of the above solution for 2–5 minutes a black precipitate or a dark coloration is the result.

OBERMEYER'S Test (*Indican in Urine*).—Precipitate the urine with a lead acetate solution (1 to 5), being careful not to add an excess of lead solution. Filter through a dry folded filter and shake the filtrate with an equal volume of fuming hydrochloric acid which contains 1–2 parts ferric chloride solution to 500 parts of the acid. Continue shaking for 1 or 2 minutes, and then add some chloroform, which takes up the indigo blue produced and is colored blue.

OBERMÜLLER'S Test (*Cholesterin*).—Fuse the cholesterin with 2 or 3 drops propionic acid anhydride in a test-tube over a small naked flame. On cooling the fused mass is first violet, then blue, green, orange, carmine, and lastly copper red.

OLIVER'S Test (*Albumin*).—Mix equal parts of sodium tungstate solution (1 to 4) and a saturated solution of citric acid, (10 to 6). The urine is floated on this solution, and in the presence of albumin a white ring is obtained at the contact of the two liquids.

PACINI'S Liquid (*Blood*).—One part corrosive sublimate, 2 parts sodium chloride, 13 parts glycerin, 113 parts distilled

water. This mixture should stand 2 months. For use mix 1 part of this solution with 3 parts water and filter.

PAVY'S Reagent (*Albumin*).—This is a practical dry reagent for albumin. It consists of small disks or plates of citric acid and sodium ferrocyanide.

PAVY'S Solution (*Glucose*).—Mix 120 c.c. of the ordinary Fehling's solution with 300 c.c. of strong ammonia (sp. gr. 0.88) and with 400 c.c. more of caustic soda solution of sp. gr. 1.14. Now dilute to 1000 c.c. with water. One hundred c.c. of this solution is reduced by glucose to the same extent as 10 c.c. of the ordinary Fehling's solution. This solution becomes decolorized by boiling with glucose solution.

PENZOLDT'S Test (*Glucose*).—Dissolve 1 part diazobenzo-sulphonic acid in 60 parts of water, and to facilitate solution add 1 or 2 drops caustic potash.

In applying this test make some of the solution to be tested strongly alkaline, and then add an equal volume of the above solution of diazobenzol sulphonic acid. It is advisable to do the same with a solution free from sugar. On allowing to stand the mixture becomes yellowish red or light claret red, then darker, and in the presence of considerable glucose it becomes dark red and opaque. The red color has a bluish shade.

PENZOLDT'S Test (*Acetone*).—A warm saturated solution of orthonitrobenzaldehyde is treated with the liquid to be tested for acetone and then made alkaline with caustic soda. In the presence of acetone the liquid first becomes yellow, then green, and lastly indigo separates, and this may be dissolved with a blue color by shaking with chloroform.

PENZOLDT and FISCHER'S Test (*Phenol*).—On treating a strongly alkaline solution of phenol with a solution of diazobenzol sulphonic acid a deep red coloration is the result.

PEPTONES. See *Hofmeister*.

PETRI'S Test (*Proteids*).—On treating a proteid or peptone solution with a solution of diazobenzol sulphonic acid only a faint yellow coloration is produced, but on making the solution alkaline with caustic alkali the solution becomes orange yellow to brown, according to concentration, and yields a red froth on shaking.

PETTENKOFER'S Test (*Bile Acids*).—A small quantity of bile in substance is dissolved in a small porcelain dish in concentrated sulphuric acid and warmed, or some of the liquid containing the bile acids is mixed with concentrated sulphuric acid, taking special care in both cases that the temperature does not rise higher than 60–70° C. Then a 10% solution of cane-sugar is added, drop by drop, continually stirring with a glass rod. The presence of bile is indicated by the production of a beautiful red liquid, whose color does not disappear at the ordinary temperature, but becomes more bluish violet in the course of a day. This red liquid shows a spectrum with two absorption-bands, the one at *F'* and the other between *D* and *E*, near *E*.

PHENOL. See *Allen*, *Berthelot*, *Davy*, *Eijkmann*, *Jacque-min*, *Landolt*, *Penzoldt* and *Fischer*.

PIOTROWSKI'S Reaction (*Proteids*), also called *Biuret* Reaction.—On heating a proteid with an excess of a concentrated solution of caustic soda and one or two drops of a dilute solution of copper sulphate a violet color is produced which deepens in tint on boiling.

PIRIA'S Test (*Tyrosin*).—Dissolve the substance in concentrated sulphuric acid and allow to stand for  $\frac{1}{2}$  an hour. Dilute with water and neutralize the solution with  $\text{BaCO}_3$  and filter. On the addition of acid-free ferric chloride to the clear filtrate a violet color is produced in the presence of tyrosin. The reaction is impeded by the presence of free acid.

POHL'S Test (*Globulins*).—He suggests to saturate the solution to one-half with ammonium sulphate, which precipitates the globulins. Filter and wash with a one half saturated solution of ammonium sulphate.

PROTEIDS. See *Adamkiewicz, Biuret, Brücke, Fröhde, Furfurol, Grigg, Liebermann, Mandel, Millon, Mulder, Michailou, Petri, Piotrowski, Reichl, Schultze, Xantho-proteid*.

PUS. See *Donné*.

RAABE'S Test (*Albumin*).—Place 1 c.c. of the liquid to be tested in a test-tube and add a small piece of trichloroacetic acid. In the presence of albumin a white zone or ring will be formed. The ring produced by uric acid is diffused and not sharply defined.

RABUTEAU'S Test (*HCl in Contents of Stomach*).—Add the filtered contents of the stomach to a solution containing 50 c.c. starch mucilage, 1 gm. potassium iodate, and 0.5 gm. potassium iodide. In the presence of free HCl it will become blue.

REES'S Test (*Albumin*).—An alcoholic solution of tannic acid precipitates small amounts of albumin.

REICHL'S Test (*Proteids*).—Add 2–3 drops of an alcoholic solution of benzaldehyde to the proteid solution, and then considerable sulphuric acid which has previously been diluted



with an equal volume of water. Lastly, add a few drops of a ferric sulphate solution, when a deep blue coloration will be produced in the cold after some time or immediately on warming. Solid proteids are also colored blue by this reaction.

REOCH'S Test (*Albumin*). See *Macwilliam*.

REOCH'S Test (*HCl in Contents of Stomach*).—A mixture of citrate of iron and quinine and potassium sulpho-cyanide is colored red by the gastric juice or contents of the stomach containing free hydrochloric acid.

REYNOLD'S Test (*Acetone*).—Precipitate  $\text{HgO}$  from a mercuric chloride solution by adding an alcoholic caustic potash solution. To this freshly precipitated  $\text{HgO}$  add the liquid to be tested for acetone, shake, and filter. In the presence of acetone the filtrate contains mercury, due to the acetone dissolving freshly precipitated  $\text{HgO}$ . The mercury is detected in the filtrate by means of ammonium sulphide, which turns black.

ROBERT'S Test (*Glucose in Urine*).—Take the specific gravity of the urine at a known temperature by means of a urinometer or pycnometer supplied with a thermometer. Now acidify slightly with tartaric acid and add a piece of yeast the size of a pea and shake. Allow to stand at the temperature of the room, or, better, at  $20\text{--}25^{\circ}\text{C}$ ., for 24–48 hours. The fermentation by this time will be finished. Now filter through a dry filter and cool to the same temperature as you took the specific gravity before fermentation. Now take the specific gravity again.

Each degree of specific gravity lost represents 1 grain of glucose to the ounce of urine, or if the number of degrees lost in specific gravity is multiplied by the factor 0.23 we

obtain the percentage of glucose or grammes per 100 c.c. of urine.

ROBERT'S Test (*Albumin*).—Allow the urine to flow on the surface of a saturated common salt solution containing 5% HCl of specific gravity 1.052. In the presence of albumin a white ring or zone will form between the two liquids.

He also suggests a mixture of 1 part strong nitric acid and 5 parts saturated magnesium sulphate solution. It is to be applied as above.

ROSENBACH'S Modification of GMELIN'S Test (*Bile Pigments*).—Filter the liquid through a very small filter. When all liquid has passed through, apply to the inside of the filter a drop of nitric acid which contains only very little nitrous acid. A pale yellow spot will be formed, which is surrounded by colored rings which are yellowish red, violet, blue, and green.

ROSENBACH'S Test (*Indigo Red or Indirubin*).—On boiling the liquid with nitric acid indigo blue is formed from the indigo red.

ROSIN'S Test (*Indigo Red or Indirubin*).—Make the liquid alkaline with sodium carbonate and extract with ether, which is colored red by the indigo red.

RUBNER'S Test (*Carbon Monoxide in Blood*).—Shake the blood for one minute with 4–5 volumes lead acetate solution. If the blood contains CO, it will retain its bright red color, while if it does not it will turn chocolate brown.

RUBNER'S Test (*Glucose*).—Treat the liquid with an excess of lead acetate, filter, and add ammonia to the filtrate until no further precipitate is produced. Warm gently, when the

precipitate produced by the ammonia will be gradually colored pink. This coloration diminishes on standing.

**SALKOWSKI'S Reaction (*Cholesterin*).**—Dissolve the substance in chloroform and then treat with an equal volume of concentrated sulphuric acid. The cholesterin solution becomes first bluish red, then gradually more violet red, while the sulphuric acid appears dark red with a greenish fluorescence. If the chloroform solution is poured into a porcelain dish, it becomes violet, then green, and finally yellow.

**SALKOWSKI'S Modification of HOPPE SEYLER'S Test (*CO in Blood*).**—Dilute the blood to be tested with 20 vols. water and add thereto an equal volume of a caustic soda solution of sp. gr. 1.34. If the blood contains carbon monoxide, the mixture will become milky in a few moments and then bright red. On standing red flakes form, which collect on the surface of the liquid. Normal blood treated in this way gives a dirty brown coloration.

**SALKOWSKI'S Reaction (*Creatinin*).**—If a few drops of a freshly prepared very dilute solution of sodium nitro-prusside are added to a dilute creatinin solution and then a few drops of caustic soda, a ruby-red liquid is obtained which quickly turns yellow (*Weyl's* reaction). If this yellow solution is treated with an excess of acetic acid and heated, the solution becomes first green and then blue, and finally a precipitate of Prussian blue is obtained.

**SALKOWSKI'S Test (*Indol*).**—Add a few drops nitric acid to the indol solution and then, drop by drop, a 2% solution of potassium nitrite. In the presence of indol a red color is produced, and lastly a red precipitate of nitroso-indol nitrate.

**SCHERER'S Test (*Inosit*).**—Evaporate the substance to dryness on a platinum foil with nitric acid and treat the residue

with ammonia and a drop of calcium chloride solution and carefully re-evaporate to dryness. With inosit a rose-red residue is obtained.

SCHERER'S Test (*Leucin*).—Carefully evaporate the leucin with nitric acid on a platinum foil. No markedly colored residue is left, but on gently warming this with a few drops of caustic soda solution a color varying from a pale yellow to a brown (depending on the purity of the leucin) is produced, and on further concentrating over the flame it agglomerates into an oily drop which rolls about on the foil.

SCHERER'S Test (*Tyrosin*).—Evaporate the substance carefully to dryness with nitric acid on a platinum foil. A beautiful yellow residue (nitro-tyrosin nitrate) is obtained, which gives a deep reddish-yellow color with caustic soda.

SCHIFF'S Test (*Uric Acid*).—Dissolve the substance in sodium carbonate and add silver nitrate solution, when a reduction of black silver oxide is obtained. If a drop of the solution of the substance in sodium carbonate is placed on a piece of filter-paper which has been previously treated with silver nitrate solution, a reduction of black silver oxide will also be formed on the paper.

SCHIFF'S Test (*Carbohydrate*).—Strips of paper are dipped in a mixture of equal volumes of glacial acetic acid and xylidin, treated with very little alcohol, and dried. On exposing such paper to the furfural vapors produced by treating glucose with sulphuric acid the paper will be colored red.

SCHIFF'S Reaction (*Cholesterin*).—Evaporate the substance in a porcelain dish over a small flame with a few drops of a mixture of 2–3 vols. concentrated hydrochloric or sulphuric acid and 1 vol. of a medium solution of ferric chloride. In

the presence of cholesterin a reddish-violet residue is first obtained and then a bluish violet.

SCHIFF'S Test (*Urea*).—Place a drop of a concentrated watery solution of furfural on the crystal of urea and then a drop of hydrochloric acid of sp. gr. 1.10. A change of color from yellow, green, blue, to purple is obtained. Allantoin gives this same reaction, but less intense and not so quickly.

SCHROEDER'S Test (*Urea*).—Place a crystal on a microscope-slide and add a solution of bromine in chloroform. Urea will not dissolve therein, but is decomposed with the development of gas.

SCHULZE'S Reagent (*Cellulose*).—Dissolve iodine to saturation in a zinc chloride solution of sp. gr. 1.8 to which 6 parts potassium iodide has been added. Cellulose turns blue with this reagent.

SCHULZE'S Test (*Cholesterin*).—Evaporate the substance to dryness on the water-bath in a porcelain dish with nitric acid. A yellow residue is obtained with cholesterin, which turns yellowish red on the addition of ammonia.

SCHULTZE'S Test (*Proteids*).—Add a few drops of a dilute cane-sugar solution and then concentrated sulphuric acid to a solution of the proteid and warm the mixture to 60° C., when a beautiful bluish-red coloration is obtained. It is important to keep the temperature at 60° C.

SCHWEITZER'S Reagent (*Cellulose*).—Sulphate of copper in solution, to which some ammonium chloride has been added, is precipitated with caustic soda; the hydrated cupric oxide thus obtained is washed and dissolved to saturation in 20% ammonia. It may also be prepared by pouring ammonia on copper turnings, the requisite oxidation of the copper being



effected by drawing a current of air through the fluid in which the turnings are immersed. Cellulose is soluble in the above reagent.

SEIDEL'S Reaction (*Inosit*).—Evaporate a small amount of the substance to dryness in a platinum crucible with a little nitric acid (sp. gr. 1.1–1.2), and treat the residue with ammonia and a few drops of a solution of strontium acetate. In the presence of inosit a greenish coloration is observed, together with a violet precipitate.

SKATOL. See *Cianician* and *Magnanin*.

SMITH'S Reaction (*Bile Pigments*).—Pour carefully over the liquid to be tested tincture of iodine, whereby a green ring appears between the two liquids.

SOLDANI'S Solution (*Glucose*).—Dissolve 15 grms. copper carbonate in 1400 c.c. water and add 416 grms. potassium bicarbonate. On heating a glucose solution with the above solution a reduction of copper suboxide is obtained.

SPIEGLER'S Test (*Albumin*).—Remove mucin from the solution by the addition of acetic acid, filter, and treat the filtrate with a solution prepared by dissolving 8 grms. mercuric chloride, 4 grms. tartaric acid, in 200 c.c. water and adding 20 grms. glycerin thereto. In the presence of albumin a white ring is obtained between the two liquids.

STOKVIS'S Test (*Bile Pigments*).—Treat 20–30 c.c. of urine with 5–10 c.c. of a solution of zinc acetate (1 to 5). The precipitate is washed on a small filter with water and then dissolved in a little ammonia. Filter, and the filtrate gives, after standing in the air, a peculiar brownish-green color, and shows the three absorption-bands of bilicyanin, the first between *C* and *D*, the second at *D*, and the third between *D* and *E*,

STOKES'S Reagent (*Reducing Oxyhaemoglobin*).—To a solution of ferrous sulphate add some citric or tartaric acid and enough ammonia to make it alkaline.

STRASSBURG'S Test (*Bile Acids*).—Treat the liquid with cane-sugar and dip a strip of filter-paper in this liquid. Dry this carefully over a gas or alcohol flame and place a drop of sulphuric acid thereon. In the presence of bile acids a red coloration is produced on the paper. The liquid must be free from albumin for this test.

STRUVE'S Test (*Blood in Urine*).—Treat the urine with ammonia or caustic potash, and then add tannin and acetic acid until the mixture has an acid reaction. In the presence of blood a dark precipitate is formed. Filter, dry, and obtain the characteristic hæmin crystals from the dry residue by the addition of ammonium chloride and glacial acetic acid. (See *Teichmann*.)

SZABO'S Test (*HCl in Contents of Stomach*).—Equal volumes of  $\frac{1}{2}\%$  solutions of ammonium sulphydric cyanide and of sodic-ferric tartrate are mixed. On adding liquid containing HCl to this solution, which is pale yellow, a brownish-red color is produced.

TANRET'S Test (*Albumin*).—Dissolve 3.32 grms. potassium iodide and 1.35 grms. mercuric chloride (4 mols. KI to 1 mol.  $\text{HgCl}_2$ ) in 20 c.c. acetic acid and dilute to 60 c.c. When this reagent is added to an albumin solution, a white precipitate is produced.

TAURIN. See *Lang*.

TEICHMANN'S Test (*Hæmin Crystals*).—Place a few particles of the dry residue on a microscope-slide, add a grain of common salt, and cover with a cover-glass. Now add some

glacial acetic acid under the cover-glass and warm gently not to boil the liquid. In the presence of blood-coloring matters the characteristic dark brown, long, rhombic crystals of hæmin are obtained. If no crystals appear after the first warming, warm again, and if necessary add some more acetic acid.

**THORMÄHLEN'S Test** (*Melanin in Urine*).—Add sodium, nitro-prusside, caustic potash, and acetic acid to the urine to be tested, and in the presence of melanin a deep blue coloration is the result.

**TROMMER'S Test** (*Glucose*).—Make the liquid strongly alkaline with caustic soda and add a not too concentrated solution of copper sulphate, drop by drop, until a little of the copper hydrate formed remains undissolved on shaking. Now warm, and in the presence of glucose a yellow reduction of hydrated suboxide of copper is first formed and then red suboxide separates even below the boiling-point. If too little copper salt has been added, the test will be yellowish brown in color, as in *Moore's* test; but if an excess of the copper salt has been added, the excess of hydrate is converted on boiling into a dark brown hydrate, which interferes with the test.

**TROUSSEAU'S Test** (*Bile Pigments*). See *Dumontpallier, Smith*.

**TYROSIN.** See *Hoffmann, Piria, Scherer, Udransky, Wurster*.

**UDRANSKY'S Test** (*Tyrosin*).—Dissolve a particle of the substance in 1 c.c. of water and add 1 drop of a 0.5% watery furfural solution and then 1 c.c. concentrated sulphuric acid. The mixture becomes faintly red. Care should be taken not to have the mixture get above 50° C.

**UDRANSKY'S Test** (*Bile Acids*).—Treat 1 c.c. of a watery or alcoholic solution of the substance with 7 drop of a 0.1%

watery solution of furfurol and allow 1 c.c. concentrated sulphuric acid to flow underneath this mixture. Care should be taken to keep the mixture cool. In the presence of bile acids a red coloration is obtained. The red color should have a shade of blue (violet) to be characteristic of bile acids.

UFFELMANN'S Test (*Lactic Acid in Contents of Stomach*).—Mix 10 c.c. of a 4% carbolic acid solution and 20 c.c. water and add a few drops ferric chloride solution, when an amethyst-blue solution is obtained. With lactic acid this solution is colored yellow.

UFFELMANN'S Test (*HCl in Contents of Stomach*).—Dip strips of filter-paper in an amyl alcohol extract of huckleberries and dry. When the contents of the stomach contains HCl, it will turn this paper pink.

ULTZMANN'S Reaction (*Bile Pigments*).—Treat the solution with caustic potash and mix, and then acidify with hydrochloric acid. The solution becomes emerald green, due to the formation of biliverdin.

UREA. See *Biuret*, *Schiff*, *Schroeder*.

URIC ACID. See *Deniges*, *Dietrich*, *v. Jaksch*, *Murexid*, *Schiff*.

UROBILIN. See *Gerhardt*.

V. D. VELDEN'S Test (*HCl in Contents of Stomach*).—A watery or alcoholic solution of Tropæolin OO, which is yellow in color, turns ruby red or deep brownish red with free hydrochloric acid. Paper moistened with the above solution may be used for the test.

VITALLI'S Test (*Bile Pigments*).—Treat the solution with a few drops of a solution of potassium nitrite and then some dilute sulphuric acid, when a beautiful green color will be obtained. This green color changes to yellow after a time, but first turns red or blue.

WEBER'S Test (*Indican in Urine*).—Treat 30 c.c. of the urine with an equal volume hydrochloric acid and 1–3 drops dilute nitric acid and heat to boiling. The solution becomes dark, and if shaken with ether, when cold, the ether will be colored red to violet, while a blue foam is observed on the top of the ether.

WEIDEL'S Reaction (*Xanthin*).—A little of the substance is dissolved in fresh chlorine water containing some nitric acid and evaporated on the water-bath to dryness. On exposing the white or yellowish residue to the vapors of ammonia, under a bell-jar, a red or purple violet color is produced.

WENDER'S Test (*Glucose*).—Dissolve 1 part commercial methylene blue in 3000 parts distilled water. On making this solution alkaline with caustic potash and heating with a glucose solution the blue color disappears and the solution becomes decolorized.

WETZEL'S Test (*CO in Blood*).—Dilute the blood with 4 vols. water and treat with 3 vols. of a 1% tannic acid solution. In the presence of carbon monoxide the blood becomes carmine red, while normal blood gradually becomes gray.

WEYL'S Reaction (*Creatinin*).—Add a few drops of a freshly prepared solution of sodium nitro-prusside to the solution of creatinin and then a few drops of caustic soda. A ruby-red liquid is obtained, which quickly turns yellow again. The solution of creatinin zinc chloride may also be used.



WITZ'S Test (*HCl in the Contents of the Stomach*).—A watery solution of methyl-anilin violet is first rendered blue, then green, and ultimately decolorized by dilute inorganic acids.

WURSTER'S Test (*Tyrosin*).—A boiling watery solution of tyrosin is colored red when treated with 1% acetic acid and a sodium nitrite solution, drop by drop.

WURSTER'S Test (*Tyrosin*).—Dissolve the tyrosin in hot water and to the hot solution add some dry chinon. The solution becomes deep ruby red, which remains for 24 hours and then passes to brown.

WURSTER'S Test (*Hydrogen Peroxide*).—Paper soaked with a solution of tetramethylparaphenylendiamine turns blue violet with hydrogen peroxide.

XANTHIN. See *Hoppe Seyler, Weidel*.

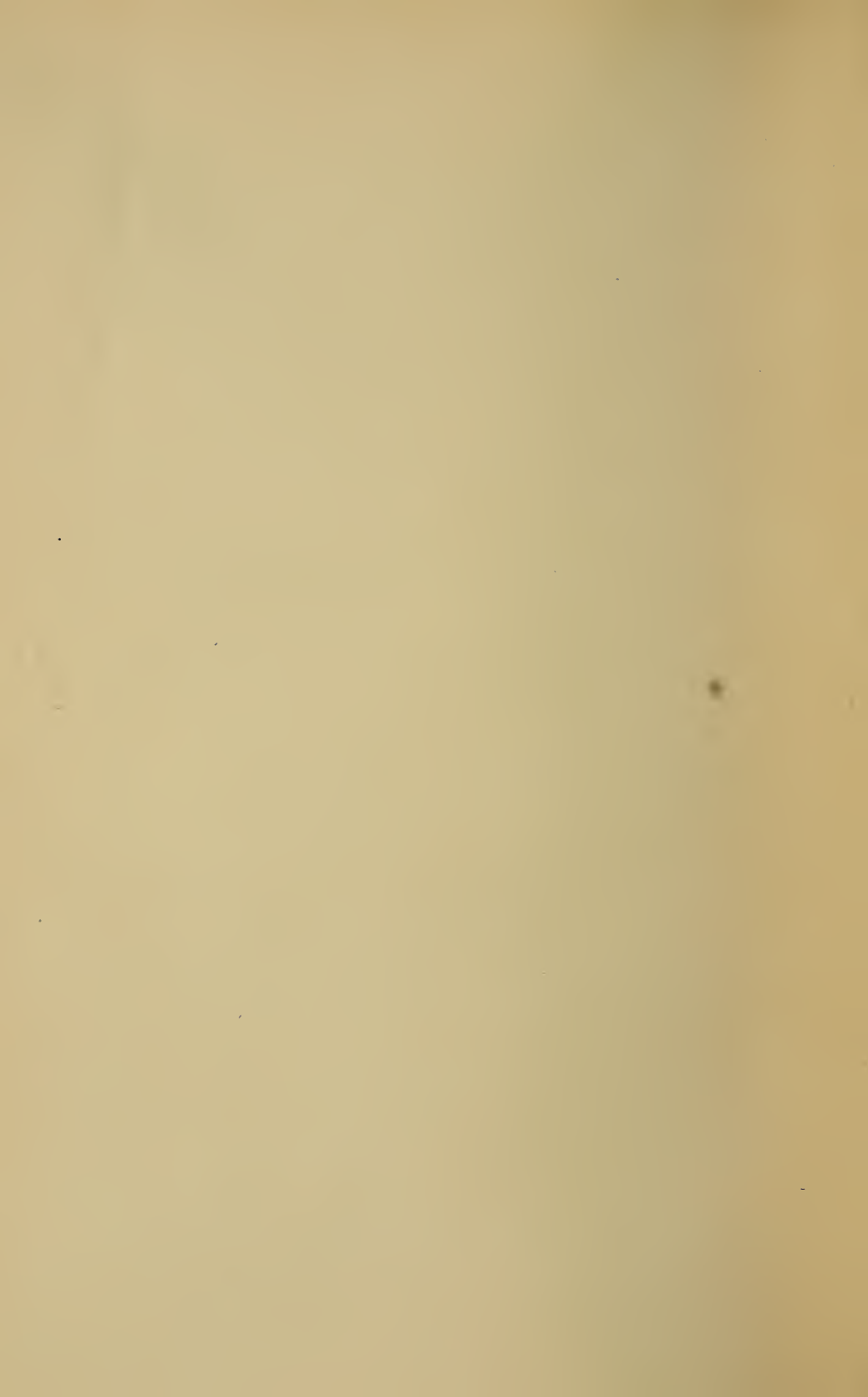
XANTHO-PROTEIC Reaction (*Proteids*). See *Mulder*.

V. ZALESKI'S Test (*Carbon Monoxide in Blood*).—Mix 2 c.c. of the blood with 2 c.c. water and 3 drops of a  $\frac{1}{3}$  saturated copper sulphate solution. With normal blood a greenish-brown precipitate is produced, while if the blood contains CO a brick-red precipitate is obtained.

ZELLER'S Test (*Melanin in Urine*).—A urine containing melanin gives, when treated with bromine water, a yellow precipitate, which gradually turns black.

ZOUCHLOS'S Test (*Albumin*).—The reagent consists of 100 parts 10% potassium sulpho-cyanide solution and 20 parts acetic acid. When this reagent is added, drop by drop, to a solution of albumin, a marked cloudiness is observed.

ZWENGER'S Test (*Cholesterin*). See Cholesterin Reactions No. 1.













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